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Rose et al.

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[54] **GLYCOPROTEIN B OF THE RFHV/KSHV
SUBFAMILY OF HERPES VIRUSES**

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Related U.S. Application Data

[63] Continuation-in-part of application No. PCT/US96/15702, Sep. 26, 1996, and a continuation-in-part of application No. 08/720,229, Sep. 26, 1996.

[60] Provisional application No. 60/004,297, Sep. 26, 1995, abandoned.

[51] **Int. Cl.**⁷ **A61K 39/245**; A61K 39/12; C12N 15/00; C12P 21/06

[52] **U.S. Cl.** **424/229.1**; 424/199.1; 424/204.1; 435/69.1; 435/69.3; 435/320.1; 435/5; 435/6; 435/7.94; 435/325; 530/350; 530/387.1; 536/23.72; 536/23.1

[58] **Field of Search** 424/199.1, 204.1, 424/229.1; 435/69.1, 320.1, 69.3, 5, 6, 7.94, 325; 530/350, 387.1; 536/23.72, 23.1

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[57] **ABSTRACT**

This invention relates to polynucleotides encoding Glycoprotein B from the RFHV/KSHV subfamily of gamma herpes viruses, three members of which are characterized in detail. DNA extracts were obtained from *Macaque nemestrina* and *Macaque mulatta* monkeys affected with retroperitoneal fibromatosis (RF), and human AIDS patients affected with Kaposi's sarcoma (KS). The extracts were amplified using consensus-degenerate oligonucleotide probes designed from known protein and DNA sequences of gamma herpes viruses. The nucleotide sequences of a 319 base pair fragment are about 76% identical between RFHV1 and KSHV, and about 60–63% identical with the closest related gamma herpes viruses outside the RFHV/KSHV subfamily. Protein sequences encoded within these fragments are about 91% identical between RFHV1 and KSHV, and <~65% identical to that of other gamma herpes viruses. The full-length KSHV Glycoprotein B sequence comprises a transmembrane domain near the N-terminus, and a plurality of potentially antigenic sites in the extracellular domain. Materials and methods are provided to characterize Glycoprotein B encoding regions of members of the RFHV/KSHV subfamily, including but not limited to RFHV1, RFHV2, and KSHV Peptides, polynucleotides, and antibodies of this invention can be used for diagnosing infection, and for eliciting an immune response against Glycoprotein B.

17 Claims, 34 Drawing Sheets

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RFHV      V Y K K N I V P Y I F K V R R Y I K I A      60
           GTGTACAAGAACAATCGTGCCGTACATTTTCAAGGTACGCAGGTACATAAAATAGCA
           *****
KSHV      GTGTACAAGAACAATCGTGCCGTATATTTTAAGGTGCGCGCTATAGGAAATTGCC      60
           V Y K K N I V P Y I F K V R R Y R K I A
(NIVPA>)  gtgtacaagaagaacatcgctgcntayatnttyaa
           (<GMTEB>) gcgccatactgtcttcgtcgt
           (<AAITB>) tcgtcgttagtgttgttttat
           (GMTEA>) gcggtatgacagaagcagcaa
                   (KYEIA>) acaaatatgagatc-
RFHV      T S V T V Y R G [M] T E [A] A I T N K Y E [I]      120
           ACATCTGTCAACGGTCTACCGGGTATGACAGAAAGCAGCAATCACAAACAATATGAGATC
           ** ***** * ** ***** * ** ***** ***** **
KSHV      ACCTCTGTCAACGGTCTACAGGGGCTTGACAGAGTCCGCCATCACCAACAAGTATGAATC      120
           T S V T V Y R G [L] T E [S] A I T N K Y E [L]
                   cgaactgtctcaggcggtagt (GLTEB)
                   (<TNKYB>) ggtagtggtgttcatacttg
                   gggcttgacagagtcgcgcat (GLTEA>)
                   (YELPA>) acaagtatgaactc-
RFHV      -cccagg (KYEIA>)
           P R P V P L Y E I S H M D S T Y Q C F S
           CCCAGGCCCGTGCCCTCTCTACGAGATCAGTCAATGGACAGCACCTACCAGTGCTTTAGT      180
           ** ***** ***** ** ***** ***** *****
KSHV      CCGAGACCCGTGCCACTCTATGAGATAAGCCACATGGACAGCACCTATCAGTGCTTTAGT      180
           P R P V P L Y E I S H M D S T Y Q C F S
           -ccgagac (YELPA>)
                   agaccggtgccactctatgarathagycayatgga (SHMDA>)
                   (<CFSSB>) acraartcr-

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FIG. 1A

RFHV

S M K I V V N G V E N T F T D R D D V N

(<TDRDB) atgcaagtggctagccctact

TCCATGAATAATTGTAGTGAACGGAGTCGAAATACGTTACCCGATCGGGATGACGTAAC 240

***** * ** ***** ** ***** ** ** ** * ** ** ** *

KSHV

S M K V N V N G V E N T F T D R D D V N

(<TDRDB) atgcaagtggctagccctact

TCCATGAAGGTAATGTCAACGGGGTAGAAAAGAGATTTACTGACAGACGATGTTAAC 240

***** * ** ***** ** ***** ** ** ** * ** ** ** *

cttccatttacagttgcccc (<VNVNB)

(<TFTDB) gtgtaaatgactgtctctgct

-agntacttctaacaacacttg (<CFSSB)

(ENTFA>) gtcaacggagtagaraaayacnttyacnga

FIG. 1B

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      (<VEGLB) gcagcttcagattgactgtt      (PVLIA>) agc-
      (VEGLA>) cccgtcgaaggtctaactgac
[K] T V F L Q P V E G L T D N I Q R Y F S
AAACCGTATTCTCCAGCCCGTCGAAGGTCTAACTGACAAACATACAAAGATACTTTAGC 300
* ** ***** ** ** ** ** ** ** ** ** ** ** ** ** ** ** **   ***** *****
ACCACAGTATTCTCCAACCAGTAGAGGGCTTACGGATAACATTCAAAGGTACTTTAGC 300
[T] T V F L Q P V E G L T D N I Q R Y F S
ccacagtattcctccaaccag (TVFLA>)      (SQPVA> ggtactttagc-
      (<DNIQB) tgnctrrttaagttcccatgaaatcg-

-caaccagtactgtactct (PVLIA>)
Q P V [L] Y [S] E P G W F P G I Y R V [G] T T
CAACCACTACTGTACTCTGAACCCGGATGGTTCCCAAGTATCTACAGGGTTGGGACAACA 360
** ** * ** * ***** ** ** ** **   ***** ** * *****
CAGCCGGTCACTACCGGGAACCCGGCTGGTTTCCCGGCATATACAGAGTTAGGACAACA 360
Q P V [I] Y [A] E P G W F P G I Y R V [R] T T
-cagccggtca (SQPVA>)
-gtcggtca (<DNIQB)
      V N C E I V D M
GTAACCTGTGAGATTGTAGACATGTT
** ***** *****
GTCAACTGTGAGATTGTAGACATGTT
V N C E I V D M
-canttracactctaactctgtacaa (<TVNCB)

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FIG. 1C

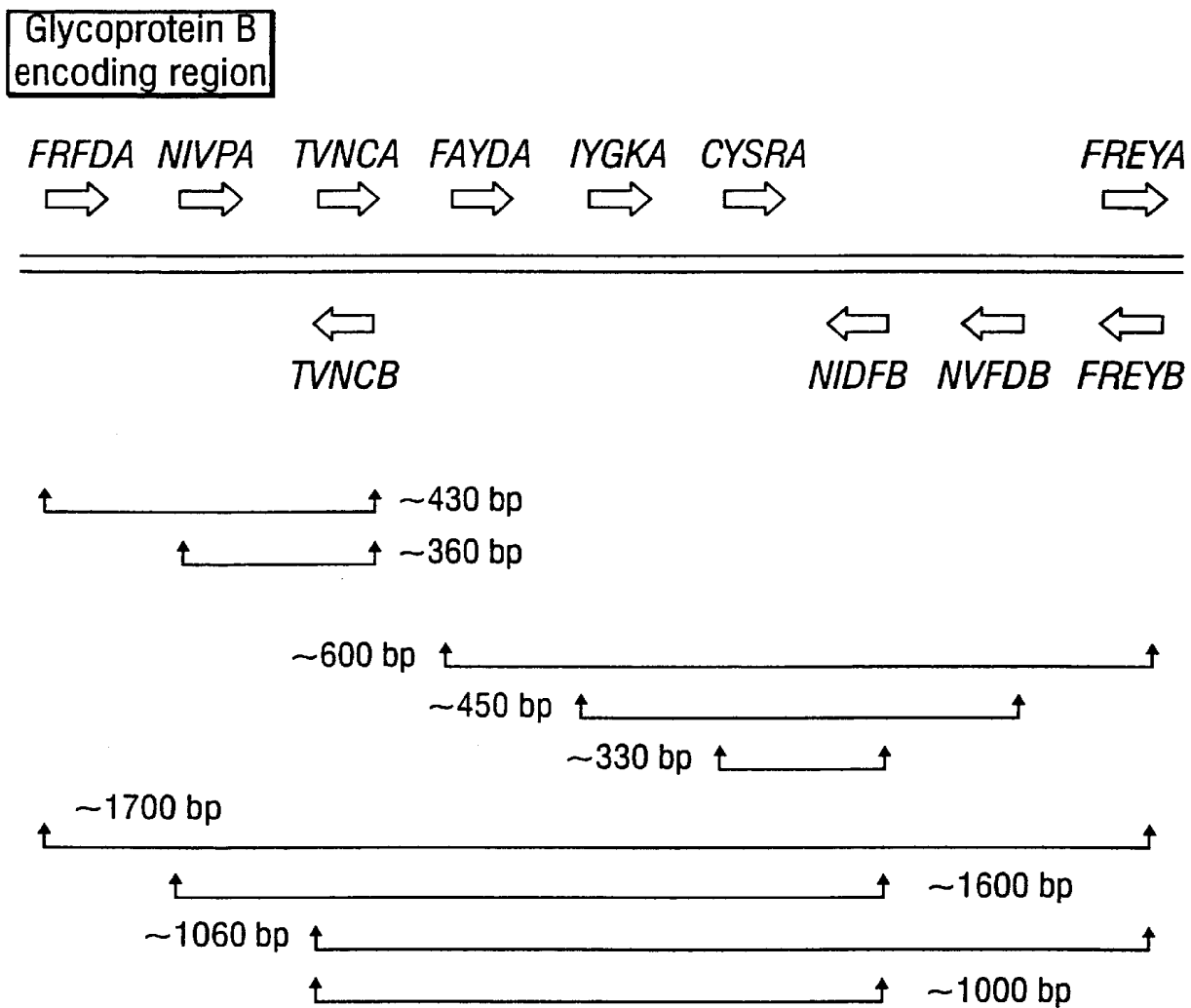
**FIG. 2**

FIG. 3A

Signal-Peptide Domain

MPT SRLRA
MVP NKHL L
MY YKT ILF
MY PTV KSM
MT RRR VLS
MS KMV VLF
MR QGA ARG

KSHV
HVS
bHV4
mHV68
EBV
hHV6
hHVS1

KSHV
HVS
bHV4
mHV68
EBV
hHV6
hHVS1

KSHV
HVS
bHV4
mHV68
EBV
hHV6
hHVS1

FIG. 3B

RFHV _{Mn}	NIVPH>	RRYIKIATSVTVYRGM--TEAAITNKYETPRPVPLY
RFHV _{Mm}		RRYRKVATPVTLYRGM--TDAAITNKYEIPRPVPLY
KSHV		PDTKDKY-HQEGILLVYKKNI VPHIFKVRRYRKIATSVTVYRGL--TESAITNKYELPRPVPLY
HVS		PSTEDKV-HKEDILLVYKKNI VPIYIFKVRRYKKITTSVRI FNGWTRGVAITNKKWELSRVAPKY
bHV4		PDTKDKK-HVEGILLVLKKNI VPIYIFKVRKYRKIATSVTVYRGW--SQAAVTNRDDISRAIPYN
mHV68		PSTQDKK-HVEGILLVYKINI VPIYIFKIRRYRKII TQLTWRGL--TTSSVTGKFEMATQAH EW
EBV		PSFGTRENHTEGLMVFKDNI IPYSEKVRSYTKIVTNIL IYNGW--YADSVTNRHEEKFSVDSY
hHV6		SPYKSNAMSEGEFFII YKTNIEITYTFPVRTYKKELTFQSSYRDV--GVVYFLDRTVMGLAMPVY
hHSV1	** * * * *	PTRPEGQNYTEGIAVVFKENI APYKFKATMYKDVTVSQVWFGH-RYSQFMGIFEDRAPVPFEE
RFHV _{Mn}		EISHMDSTYQCFSMKI VVNGVENTFTDRDDVNKT VFLQPV EGLTDNIQRYFSQ--PVL YSEPG
RFHV _{Mm}		EISHMDSTYQCFSMKI VVNGVENTFTGRDDVNKSVFLQPV EGLTDNIKRYFSQ--PVL YSEPG
KSHV		EISHMDSTYQCFSMKVNVNGVENTFTDRDDVN TTVFLQPV EGLTDNIQRYFSQ--PVI YAEPPG
HVS		EIDIMDKTYQCHNCMQIEVNGMLNSYYDRDGN NKTVDLKPVDGLTGATRYISQ--PKVFADPG
bHV4		EISMIDRTYHCF SAMATVINGILNTYIDRDS ENKSVPLQPVAGLTENINRYFSQ--PLI YAEPPG
mHV68		EVGDFDSIYQCYN SATMVVNNVRQVYVDRDGVNKT VNI RPDGLTGNIQRYFSQ--PTL YSEPG
EBV		EDTQMDTIYQCYN AVKMTKDGLTRVYVDRDGVNIT VNLKPTGGLANGVRRYASQ--TEL YDAPG
hHV6		EANLVNSHAQCYSAVAMKRPDGT VFSAFHEDNNKNNTL NLFPLNFKSITNKRFITTTKEPYFARG
hHSV1	* * *	VIDKINAKGVCRSTAKYVRNNLETTAFHRDDHETDMELKPANAATRTSRGWH--TTDLKYN-PS

FIG. 3C

REHVM_n WFPGIYRVG
 REHVM_m WFPGIYRVR
 KSHV WFPGIYRVRTTVNCEIVDMIARSAEPYNYFVTS₁LGDTVEVSPFCYNES--CSTTPSNKN--GLS

 HVS WLWGYRTRTTVNCEIVDMFARSADPYTYFVTALGDTVEVSPFCDVDNS--CPNAT-----DVLS
 bHV4 WFPGIYRVRTTVNCEIVDMYARSVEPYTHFITALGDTIEISPCH--NNSQCTTGNSTSRDATK
 mHV68 WMPGFYRVRTTVNCEIVDMVARSM₁DPYNYIATALGDSLELSPFQTFDNTSQCTAPKRA--DMRV
 EBV WLIWYRTRTTVNCLITDMMAKSNSPFDFEFTTGTQTVEMSPFYDGKNKETFHE-----RADS
 hHV6 P-LWLYSTSTSLNCIVTEATAKAKYPFSYFALT₁TGEIVEGSPFFNGSNGKHFAE₁PLEK--LTIL
 hHSV1 RVEAFHRYGTTVN₁NCIVEEVDARSVYPYDEFVLATGDFVYN₁SPFYGTREGSHT₁ETS₁YAADR₁FKQ
 * * * * *
 KSHV QQVVLNHTVV₁TYSDRGTSPTPQNRIFVETGAYT₁LSWASESKTTAVCP₁LALWKT₁FPRSIQTTHED

 HVS VQIDLNHTVVDYGNRATSQQHKKRIFAHTLDYSVSWEAVNKSASVC₁SMVFWKSFORAIQT₁EHDL
 bHV4 VWIEENHQTVDY-ERRGHP₁TKDKRIFLKDEEY₁TSWKAEDRERAI₁CD₁FVIWKT₁FPRAIQT₁IHNE
 mHV68 REVK-NYKFVDYNNRGTA₁PAGQSR₁T₁FLET₁PSAT₁YSWKTAT₁RQ₁TAT₁CD₁LVHWKT₁FPRAIQT₁AHEH
 EBV FHVRTNYKIVDYDNRGTNPQGERRAFLDKGT₁Y₁LSW₁KL₁ENRTA-YCPLQHWQT₁FDST₁IATETGK
 hHV6 ENYTMIEDLMNG-MNGAT₁TLVRKIA₁FL₁EKA₁DTL₁FSWEIKEENESVC₁MLKHWTT₁VTHGLRAETDE
 hHSV1 -VDG₁FYARDL₁TTKARATA₁PT₁TRN--LLTTPK₁FTVAWD₁WVPKRPSV₁CTMT₁KWQ₁EVDEM₁LRSEGGG
 * * * * *
 KSHV SFHFVANEITATFTAPLTP---VANFTD₁YSCLTSDINT₁TTLNA-SKAKLASTHVPNGTVQYFHT
 HVS TYHFIANEITAGFSTVKEP---LANFTSDYNCLMTHINT₁TLED-KIARVNNHTHPNGTAEY₁YQT
 bHV4 SFHFVANEV₁TASFLTSNQEETELRG₁TEILNC₁MNSTINET₁LEE-TVKKFNKSHIRDGEVKY₁YKT
 mHV68 SYHFVANEV₁TATFNTPLTE---VENFTST₁YS₁CVSDQINK₁TISE-YIQKLNNSYVASGKTQYF₁KT
 EBV SIAVFTDEGTSSFVTNTTV---GIELPDAFK₁CIEEQVNKT₁MEKYEAVQDRYTKGQEAITYFIT
 hHV6 TYHFI₁SKELTA₁AFVAPKES---LNLTDPKQT₁CIKDEFEKI₁NEVYMSDYN₁DTYSMNGSYQ₁IEK₁T
 hHSV1 SFR₁ESSDAISTTFTTNL₁TE---YPLSRV₁DLGD₁CI₁GKDARDAMDRI₁FARRYNATHIKVGPQY₁YLA
 *

FIG. 3D

KSHV	TGGLYLVWQPM SAINLTHAQD SGNPTSSPPPSASPM TTSARRK-----
HVS	EGGMILVWQPLIAIELEEA MLEATTSPTVTPSAPTSSSR SKRAI-----
bHV4	NGGLFLIWQAMKPLNLSE-----HTNYTIERNNKTGNKSRQK-----
mHV68	DGNLYLIWQPLEHPEIEDIDEDSDPEP-TPAPPKSTRRKREAA-----
EBV	SGGLLLAWLPLTPRSLATVKNLTTELTTPTSSPPSSPPAPSAARGSTPAAVLRRRRRDAGNAT
hHV6	TGDLILIWQPLVQKSL--MFLEQGSEKIRRRRDVVDVKS R HDI-----
hHVS1	NGGFLIAYQPLLSNTLAE LYVREHLREQSRKPPNP T P P P P G A S-----*
KSHV	RRSASTAAAGGGSTDNLSY TQLQFAYDKLRDGINQVLEELSRAWC REQVRDNIMWYELSKINP
HVS	-----RSIRDVSAGSENNVFLSQIQAYDKLRQSI NNVL EE LAITWC REQVRQTMVWYELAKINP
bHV4	-----RSVDTKTFQAKGLSTAQVQYAYDHLRTSMNHILEELTKTWCREQKKDNIMWYELSKINP
mHV68	-DNGNSTSEVSKGSENPLITAQIQFAYDKLTTSVNNVLEELSRAWC REQVRDTIMWYELSKVNP
EBV	TPVPPTAPGKSLGTLNNPATVQIQFAYDSLRRQINRM LGDLARAWC LEQKRQNMVLRELTKINP
hHV6	-----LYVQLQLYDLTKDYINDALGNLAESWC LDQKRTITMLHEL SKISP
hHVS1	-----ANASVERIKTTS SIEFARLQFTYNHIQRHVNDMLGRVAIAWC ELQNHELTTLWNEARKLNP

Fig. 3E

KSHV	TSVMTAIYGRPVSAKFVGDAISVTECINVDQSSVNIHKSLRTNSK-----DVCYARPLBTFFKF	<i>CYSRA</i> >
HVS	TSVMTAIYKGPVSRKALGDVIVSVECINVDQSSVSIHKSLKTENN-----DICYSRPPVTFKF	
bbHV4	VSVMAAIYKGPVAVKAMGDAFMVSECINVDQASVNIHKSMRTDDP-----KVCYSRPLVTFKF	
mmHV68	TSVMSAIYKGPVAARYVGDAISVTDCIYVDQSSVNIHQSLRLQHD-----KTTCYSRPLVTFKF	
EBV	TTVMSSIIYGKAVAAKRLGDVIVSQCVPNQATVTLRKSMRVPGS-----ETMCYSRPLVSFSF	
hhHV6	SSIIVSEVYGRPISAQLHGDVLAISKCIENVQSSVQLHKSMRVVDAKGVRSCTMCYNRPLVTFFSF	
hhHVS1	NAIASATVGRRV SARMLGDVMAVSTCVPVADNVIVQNSMRISSR-----PGACYSRPLVSFRY	*** ** *
KSHV	LNSS-NLFTGQLGARNEIILTNQVETCKDTCEHYFITRNETLVYKDYAYLRTINTTIDISTLNT	
HVS	VNSS-QLFKGQLGARNEIILSESIVENCHQNAETFTAKNETYHFKNYVHVETLPVNNISTLDT	
bbHV4	VNST-ATFRGQLGTRNEIILTNTHVETCRPTADHYFFVKNMTHYFKDYKEVKMTMDTNISTLDT	
mmHV68	INST-DPLTGQLGPRKEIILSNTNIETCKDESEHYFIBGEYIYYKNIYIFEEKNLSSIATLFT	
EBV	INDT-KTYEGQLGTDNEIFLTCKMTEVCQATSQYYFQSGNEIHVYNDYHHFKTIELDGIATLQT	
hhHV6	VNSTPEVVPQGLGDNEILLGDHRTEECEIPSTKIFLSGNHAHVTDYHTNSTPIEDIEVLDA	
hhHVS1	EDQG-PLVEGQVGENNELRLTRDAIEPCTVGHRRYFTFGGGYVYFEEYAYSHQLSRADITTVST	*** ** *
KSHV	FIALNLSFIQNIIDFKAIELYSSAEKRLASSVFDLETMFREYNYTHRLAGLREDLNTIDMNKE	<NIDFB <NFDB <FREYB FREYA>
HVS	FLALNLTFIENIDFKAVELYSSGERKLA-NVFDLETMFREYNYAQSI SGLRKDFDNSQRNDRD	
bbHV4	FLTNLTFIDNIDFKTVELYSETERKMA-SALDLETMFREYNYTQKLASLREDLNTIDLNRD	
mmHV68	FIALNISFIENIDFKTVELYSSSTERKCLASSVFDIESMFREYNYTYSLAGIKKDL,DNTIDYNRD	
EBV	FISLKIDPLENADFKVLDLYSPDELSRA-NVFDLEGIFREYNFQAQNIAGLRKDL,DNAVSNGRN	
hhHV6	FIRLKIDPLENADFKVLDLYSPDELSRA-NVFDLENILREYNSKSAlyTIEAKI-----AANTP	
hhHVS1	FIDLNIITMLEDHEFVPLEVYTRHEIKDSGLLDYTEVQRRNQHLDRFADIDTVIHADANAAMV-	*** ** *

Membrane-spanning domain

FIG. 3F

Membrane-spanning domain				
KSHV	RFVRDLSEIVADLGGIGKTVVNVASSVVTLCGSLVTGFIN-----FIKHP	LGGM	IIIVIA	
HVS	RIIQDFSEILADLSIGKVI VNVASGAFSLFGGIVTGILN-----FIKNP	LGGM	FTFL	LIGA
bHV4	RLVKDLSEMMADLDIGKVVVNTFSGIVTVFGSIVGGFVS-----FFTNP	IGGV	TIILL	LIV
mHV68	RLVQDLSDMADLDIGR SVNVVSSVVTFFSSIVTGFIK-----FFTNP	LGGI	FILLI	IGG
EBV	QFVDGLGELMDSLGSVGQDITNLVSTVGGLFSSLSBSGFIS-----FLKNP	FGG	MLI	LAIV
hHV6	SYVNGINSEFLQGLGAIGTGLGSVISVTAGALGDIVGVVS-----FLKNP	FGG	MLI	LAIV
hHVS1	IHADANAAMFAGLGAFFEGMGDLGRAVGKVV* [*] MGIVGVVSAVSGVSSFMSNP* [*] * [*]	FGAL	AVGL	LVLA
KSHV	IIILIIEMISRRNTNTIAQAPVKMIYPDVDRRAPPSSGGAP-----TREEIKNILLGMHQLQQE			
HVS	VIILVILIVRRRTNMSQAPIRMIYPDVEKSKSTVTP-----MEPETIKQILLGMHNMQQE			
bHV4	VVFVVFIVSRRRTNNMNEAPIKMIYPNIDKASEQENIQP-----LPGEEIKRILLGMHQLQQS			
mHV68	IIFLVVILNRRNSQFHDAPIKMLYPSVENYAAEQAPPYSASPPIAIDKEEIKRILLGMHGVHQE			
EBV	VVILVISITRRTRQMSQQPVQMLYPGIDELAQQHASGEGPGINP-ISKTELQAIMLALHEQNQE			
hHV6	VVVIIVVFEVRQRHVLSKPIDMMFPYATNPVTTVSSVTGTTVVKTPSVKDVDDGGTSVAVSEKEE			
hHVS1	GLAAAFEF* [*] FRYVMRLQSNP* [*] KALYPLTTTKELKNPTNPDASGEGEGGDFDEAKLAEAREMIRYM			

FIG. 3G	KSHV	ERQKADDLKKSTPSVFQRTANG-LRQRLGYKPLTQSLDISPETGE
HVS	bHV4	AYKKKEEQRAARPSIFRQAAETFLR-KRSGYKQISTEDKIV
mHV68	EBV	EHGKSEEEAASHKPGLFQLLGDGLQLLRRRGYTR-LPTFDPSPGNDTSETHQKYV
hHV6	hHVS1	EKEAQKQLTNSGPTLWQK-ATGFLNRNRKGYSQ-LPLEDESTSL
		QKRAAQRAAGPSVASRALQAARDRFPGLRRRRYHDPETAALLGEAETEF
		ADVSGQVSDDEYSQEAALKMLKAIKSLDES YRR-KPSSSESHASKPSLIDRIRYRGYKSVNVEEA
		ALVSAMERTEHKAKKGTSA LLSAKVTDVMVRKRRTNYTQVPNKDGDADDDL

FIG. 4

EBV
 SHV1
 mHV68
 bHV4
 5'-gctgttcagatttgacttagaymanmcntgycc-3'
 FRFDA 256-fold 33mer>

FIG. 5

EBV
 SHV1
 mHV68
 bHV4
 5'-gtgtacaagaagaacatcgtgcntayatnttyaa-3'
 NIVPA 64-fold 32mer>
 5'-gtgtacaagaagaacatcgtgcc-3'
 NIVPASQ 23MER>

FIG. 6

EBV
 SHV1
 mHV68
 bHV4
 5'-tggacttacaggactcgaacnacngtnaaytg-3'
 TVNCA 128-fold 32mer>
 3'-tgntgncanttracactctaacaatctgtacaa-5'
 <TVNCB 128-fold 32mer
 3'-acactctaacaatctgtacaa-5'
 <TVNCBSQ 20mer

FIG. 7

EBV
SHV1
mHV68
bHV4

AACAATCCCGCCACCGTCCAGATCCAATTTGCCCTACGAC
AATAATGTGTTTCTATCACAATAACAATATGCATATGAT
AATCCGCTCATACGGCCCAAAATTCATTTGCCCTATGAC
AAGGCCCTGTCCACTGCCCAGGTTCAATATGCCCTATGAC

5'-aataacctctttacggcccaaatcartwygcntayga-3'
FAYDA 64-fold 38mer>

FIG. 8

EBV
SHV1
mHV68
bHV4

AATCCAACCAACCGTCATGTCCAGCATCTACGGTAAGCGGTG
AATCCAACAAGTGTATGACAGCAATATATGGAACCTGTC
AACCCTACGAGTGTGATGCTGCCATTTATGGAAAGCCCTGTC
AACCAGTGAGTGTCAATGGCAGCCATTTATGGGAACCTGTG

5'-ccaacgagtgatgtcagccatttayggnaarccngt-3'
IYGKA 64-fold 38mer>
5'-ccaacgagtgatgtcagcc-3'
ITGKASQ 21mer>

FIG. 9

EBV
SHV1
mHV68
bHV4

TGCTACTCGGCCCCCTGGTGTCTTCAGCTTTATCAACGAC
TGCTATTACAGGCCCTCCAGTTACATTTAAATTTGTTAACAGT
TGCTACTCGAGACCTAGAGTCACCTTCAAATTTATAAACAGT
TGTTACTCCAGACCCCCTGGTCACATTTAAATTTGTGAATAGT

5'-tgctactcgcgacctctagtcaccttyaarttyrtnaa-3'
CYSRA 64-fold 38mer>
5'-tgctactcgcgacctctagtcacc-3'
CYSRASQ 24mer>

FIG. 10

EBV AACATTGACTTTGCCTCCCTGGAGCTGTACTCACGGGACGAACAGCGT
 SHV1 AATATTGACTTTAAAGCTGTTGAATTGTATTCAAGTGGAGAGAGAAAG
 mHV68 AATATCGACTTCAAACAAGTAGAACTGTACTCCTCTACTGAAAGGAAA
 bHV4 AATATAGATTTCAAGACAGTGAACACTTTACAGTGAGACTGAAAGAAAG
 3'-ttrtadcttraarttytgtcaccttgacatgagggcca-3'
 <NIDFB 48-fold 36mer>
 3'-tgtcaccttgacatgagggcca-5'
 <NIDFBSQ 21mer>

FIG. 11

EBV AACGTCTTTGACCTGGAGGGCATCTTCCGGGAGTACAAC'TCCAGGCGCAAAAC
 SHV1 AACGTGTTTGATTAGAGACTATGTTTAGAGAAATATAACTATTACGCTCAGAGT
 mHV68 AGCGTCTTTGATATAGAAATCCATGTTTAGGGAATATAACTATTACACCTACAGC
 bHV4 AGTGCCCTCGACCTGGAGACCATGTTTAGAGAGTATAATTACTACACACAGAAG
 5'-tttgacctggagactatgttymngnartya-3'
 FREYA 64-fold 32mer>
 3'tacaartcycyatrtrtgatgatgtgggtctcg-5'
 <FREYB 16-fold 33mer>
 3'-ttrcanaarctrhgacctctcgtaagaagctct-5'
 <NVFDB 32-fold 32mer>

FIG. 12

EBV TTCATCTCCTTCTTCAAAAACCCCTTCGGGGCATGCTC
 SHV1 ATATTAAATTTTATTAAAAATCCTTTAGGTGGCATGTTT
 mHV68 TTCAATTAAATTTCTTTACCAACCCCTCTAGGGGGAATATTC
 bHV4 TTTGTCAAGTTTTTTCACAAAACCCCATTTGGGGCGGTGACG
 5'-accttcatcaaaaaatcccttngnggnatgyt-3'
 GGMA 128-fold 32mer>

FIG. 13A

V R R Y R K I A T S V T V Y R G L T E
 KSHV GGTGGCGCTATAGGAAATTCACCTCTGTACGGTCTACAGGGCTTGACAGAG--
 RFHV ...A..CA.G..C.TA...A..A..A...C.C..TA...A--
 bHV4 A...A..AAA...A...A...A..G..A..T...A..G.G.T.CC...--
 sHV1 A..CA.AA.A..C.AA...CA.A..A..A..CGTA.T.TT.AT...G..TAGAGA
 eHV2 T..CA.AAAG...G..CATG...GAC...CA...A...T.G..GC...--
 mHV68 AA.CA..A.A...A..A..AATT..TCAAC.G..CA...GGC.A...C.A..CACT--
 hEBV ...C..CTC...C.CC..G..A.TG...AACA.TCTCA...AT...G.TAC.C.--

S A I T N K Y E L P R P V P L Y E I S
 KSHV ----TCCGCCATCACCAACAAGTATGAACCTCCGAGACCCGTGCCACTCTATGAGATAAG
 RFHV ----G.A..A...A...A...GA...C.G...T...C...C...
 bHV4 ----G.A..TG.T...T.G.G...TA..AGC...G..A.A..CTATA...A..TTC
 sHV1 AGGTGTT..T..T..A...A.GG...TT.T...G.T..T...AAA...GA
 eHV2 ----GAT...A..A..C..C.CACGAGGAGCTACG...C..C.G..C...G.CCA
 mHV68 ----AGTT.AG...TGGT..A.T...A.GG.C.CT.AG.CC.ACGAG.GG..AG.GG.
 hEBV ----GA.T..G.G...CG.C.C..GGAGAA.TTCT...TGACAG...C..A.CTGA

H M D S T Y Q C F S S M K V N V N G V E
 KSHV CCACATGGACAGCACCTATCAGTGCTTTAGTTCCATGAAGGTAATGTCAACGGGGTAGA
 RFHV T.....C.....AA.TGTA..G..C..A..C..
 bHV4 AATG..A..T..G...T..T..CTC.G.T...GCAAC.GTCA.T..T..A.TCT
 sHV1 TATT...T.AG..T..C..A..TCAT.A..G...C..A..G.A..A...AA.GTT
 eHV2 GATG...CA.TAT...CG..G.AC...C..C.AGGG...CAC.T
 mHV68 .G..T.T...T...AC.A.AG.GCC.CCA.GGTG..A...AAC...CAG
 hEBV ...G...T.C..T...C...AC.ACG.GG.C...A.G.CAAAG.T...C.GAC

FIG. 13B

	N	T	F	T	D	R	D	D	V	N	T	T	V	F	L	Q	P	V	E	G
KSHV	AAACACATTTACTGACAGACACGATGTTAACACACACAGTATTCCTCCAACCACTAGAGGG																			
RFHV	...T..G..C..C..TC.G..T..C..A...AA..C...T...G..C..C..A...																			
bHV4	G...C.A..TA...G..TTC..AA..T.AGT..T..TCC...G...G.CC...																			
SHV1	...TT.T.ACTA...T.GAAA...AA..T...GA.T.AA.G..T...T...																			
eHV2	C...C.ACTA...G...GGTGG...GAG..C.CC...A...G.CC..T...																			
mHV68	C.GGTG.A.GTG...T.GG..C..T.AA..T..GAA.A.A.GC..T..T..T...																			
hEBV	GCG.GTG.A.GTA...C.C...GA...T...C..CAA...AA.G..CACC.G...																			

	L	T	D	N	I	Q	R	Y	F	S	Q	P	V	I	Y	A	E	P	G	W
KSHV	GCTTACGGATAACATTCAAAGGTACTTTTAGCCAGCCGGTCATCTACGCGGAACCCGGCTG																			
RFHV	T..A..T..C...A...A...A...A...A..AC.G..T.T...A...A...																			
bHV4	A..G..T..G...TAA.C.A...T..T..A..TC...A..T..A...T...T...																			
SHV1	T..A...G.GCA.T.AC...A...A.T...A..TAAAG.T.TT..T..T...T...																			
eHV2	T..C..CTC..G..TAACGC.C..TCAG..T..A..A.AGG.G...CACC...A.AAA																			
mHV68	T..A..A.GG..T..C...A...T...T...CAC.C.T..TT.A...T..T...T...																			
hEBV	C..GG.CA.CGGG.G.GCC.C...GCC...A...AGC...T.AC.CC...G...																			

	F	P	G	I	Y	R	V	R
KSHV	GT'TTCCCGCATATACAGAGTTAGG							
RFHV	...C..A..T..C...G...G...							
bHV4	...A..G..T...T...G..A							
SHV1	.C.ATGG..A.CT...GAC.C.A							
eHV2	CC.GTTGT.GTCT...C.ACA..A							
mHV68	.A.G..T...T..TC.T...C.A							
hEBV	...GATAT.G.CT...ACA..A							

FIG. 14

KSHV	VRRYRKIATSVTVYRGLTES--AITNKYELPRPVPLYEISHMDSTYQCFSSMKVNVNGVE			
RFHV	I.....M..A--.....I.....IV.....	<u>VTVYRG</u>	<u>AITNKYE</u>	<u>SHMDSTY</u>
bHV4	K.....WSQA--V..RDDIS.AI.YN..MI.R.H..A.ATVI..IL	rgltesa		<u>VE-</u>
shV1	K..T...RIFN.W.REGV.....W..S.A.K..DIM.K...HNC.QIE...ML	rgmteaa		
mHV68	I.....I.QL.IW....T.--SV.G.F.MATQAHEW.VGDF..I...YN.ATMV..N.R			
eHV2	K...M..T.I.K.WS.D--...QHTRSYA....VQM..HY.....AVQ..EG.HV			
hEBV	S.T..V.NILI.N.WYAD--SV..RH.EKFS.DS..TDQ..TI...YNAV.MTKD.LT			
KSHV	NTFTDRDDVNTTVFLQPVEGLTDNIQRYFSQPVIIYAEPGWFPGIYRVR			
RFHVK.....L.S.....G			
bHV4	..YI...SE.KS.P....A...E.N.....L.....			
shV1	.SYY...GN.K..D.K..D...GA.T..I...KVF.D...LW.T..T.			
mHV68	QVYV...G..K..NIR..D...G.....TL.S....M..F.....			
eHV2	..YY...GW.E.A..K.AD...SS.T..Q...EV..T.RNLLWS.TT.			
hEBV	RVYV...G..I..N.K.TG..ANGVR..A..TEL.DA...LIWT..T.			
	<u>-NTFTD</u>	<u>TVFLOPV</u>	<u>TDNIORY</u>	<u>pviyaep</u>
				<u>pvylysep</u>
				RFYSQP

(-) Deletions
(.) Amino acids identical to KSHV

FIG. 15

KSHV VRRYRKIATSVTVYRGLTES--AITNKYELPRVPPLYEISHMDSTYQCFSSMKVN
 RFHV VRRYIKIATSVTVYRGMTEA--AITNKYEIPRPVPLYEISHMDSTYQCFSSMKIV
 bHV4 VRKYRKIATSVTVYRGWSQA--AVTNRDDISRAIPYNEISMIDRTYHCFSAMATV
 sHV1 VRRYKKITTSVRIENGWTRREGVAITNKWELSRVAPKYEIDIMDKTYQCHNCMQIE
 eHV2 VRKYRKIMTSTTIYKGWSED--AITNQHTRSAYVPLYEVQMMDDHYQCFSAVQVN
 mHV68 IRRYRKIIITQLTIWRLTTS--SVTGKFEMATQAHEWEVGDSDSIYQCYNSATMV
 hEBV VRSYTKIIVTNILINYGWYAD--SVTNRHEEKFSVDSYETDQMDTIYQCYNAVKMT
 hCMV VRVYQKVLTFRRSYAYIHTT--YLLGSNTEYVAPPMEIHHINSHSQCYSSYSRV
 hHHV6 VRTYKKELTFQSSYRDVGCV--YFLDRTEMLAMPVYEANLVNSHAQCYSAVAMK
 hVZV ATVYKDVIVSTAWAGSSYT-QITNRYADRVPIPVSEITDTIDKFGKCSSKATYV
 sHVS A8 ATMYKDVTVSQVWFCHRY-S-QFMGIFEDRAPVPFEEVMDKINAKGVCNSTAKYV
 hHSV1 ATMYKDVTVSQVWFCHRY-S-QFMGIFEDRAPVPFEEVIDKINAKGVCNSTAKYV

KSHV V-NGVENTFTDRDDD-N'TTVFLQPVEGLTDNIQRYFSQPVIYAEPCWFPGIYRVR
 RFHV V-NGVENTFTDRDDV-NKTVFLQPVEGLTDNIQRYFSQPVLVLYSEPCWFPGIYRVG
 bHV4 I-NGILNTYIDRDSE-NKSVPLQPVAGLTENINRYFSQPLIYAEPCWFPGIYRVR
 sHV1 V-NGMLNSYYDRDGN-NKTVDLKPVDGLTGATRIYSQPKVFADPGWLWGTYRTR
 eHV2 E-GGHVNTYYDRDGV-NETAFLEKPADGLYSSITRYQSQPEVYATPRNLLWSYTR
 mHV68 V-NNVRQVYVDRDGV-NKTVNIRPVDGLTGNIQRYFSQPTLYSEPGWMPGFYRVR
 hEBV K-DGLTRVYVDRDGV-NITVNLKPTGGLANGVRRYASQTELYDAPGWLWYRTR
 hCMV I-AGTVFVAYHRDSYENKTMQLMPDDYSNTHSTRYVTVKDQWHSRGSTW-LYRET
 hHHV6 RPDGTVFSAFHEDNNKNNTLNLFPLEFKSITNKRFTITKEPYFARGPLW-LYSTS
 hVZV R-NNHKVEAFNEDKN-PQDMPLIASKYNVSGSKAWHTTNDTYMVAG-TPGTYRTG
 sHVS A8 R-NNMESTAFHRDDH-ESDMALKPAKAATRTSRGWHTTDLKYNPARVEAFHRYGT
 sHSV1 R-NNLETTAFHRDDH-ETDMELKPANAATRTSRGWHTTDLKYNPSRVEAFHRYGT

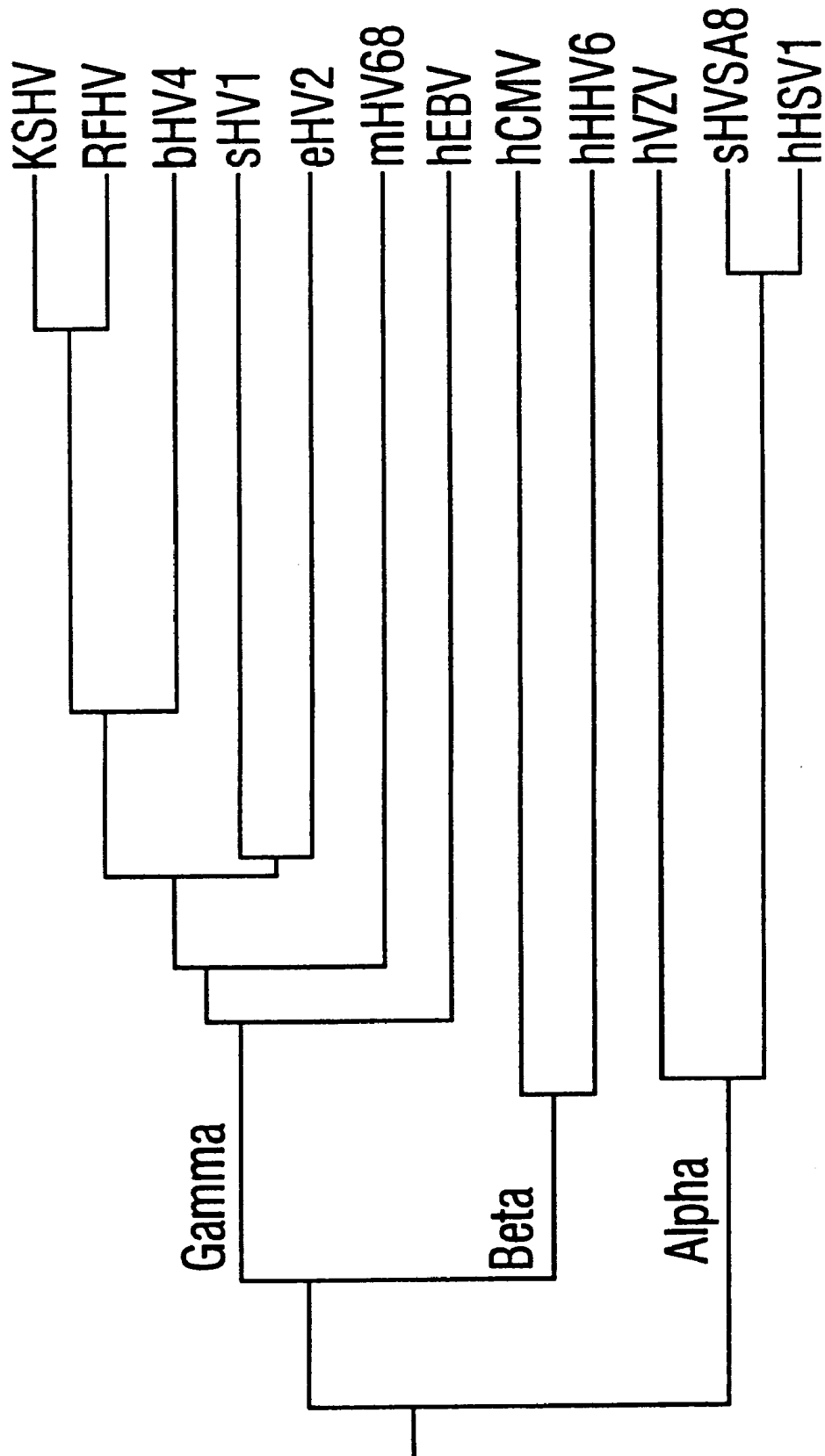


FIG. 16

FIG. 17A

KSHV	GGTGGCGCTATAGGAAATTGCCACCTCTGTACGGTCTACAGGGCTTGACAGAG--		
RFHV	...A..CA.G..C.TA....A..A..A.....C.C..TA.....A--		
bHV4	A...A..AAA.....A.....A..G..A..T....A..G.G.T.CC...--		
sHV1	A..CA.AA.A..C.AA....CA.A..A..A..CGTA.T.TT.AT...G...TAGAGA		
eHV2	T..CA.AAAG.....G..CATG.....GAC...CA.....A...T.G..GC...--		
mHV68	AA.CA..A.A....A..A..AATT..TCAAC.G..CA...GGC.A...C.A..CACT--		
hEBV	...C..CTC...C.CC..G..A.TG...AACA.TCTCA.....AT....G.TAC.C.--		
KSHV	----TCCGCCATCACCAACAAGTATGAACCTCCCGAGACCCGTGCCACTCTATGAGATAAG		
RFHV	----G.A..A....A....A....GA....C..G.....C.....C....C...		
bHV4	----G.A..TG.T....T.G.G....TA..AGC...G..A.A..CTATA....A..TTC		
sHV1	AGGTGTT..T..T..A....A.GG.....TT.T...G.T..T...AAA.....GA		
eHV2	----GAT....A..A....C..C.CACGAGGAGCTACG....C..C..G..C...G.CCA		
mHV68	----AGTT.AG....TGGT..A.T....A.GG.C.CT.AG.CC.ACGAG.GG..AG.GG.		
hEBV	----GA..T..G.G.....CG.C.C..GGAGAA.TTCT...TGACAG...C..A..CTGA	(SHMDA>)	agaccctgccactctatgarathag-
KSHV	CCACATGGACAGCACCTATCAGTGCTTTAGTTCATGAAGGTAATGTCAACGGGTAGA		
RFHV	T.....C.....C.....AA.TGTA..G..C..A..C..		
bHV4	AATG..A..T..G.....T..T..CTC.G.T...GCAAC.GTCA.T..T...A.TCT		
sHV1	TATT....T.AG..T..C..A..TCAT.A..G....C..A..G..A..A....AA.GTT		
eHV2	GATG.....CA.TAT.....CG..G.AC....C..C.AGGGG...CAC.T		
mHV68	.G..T.T.....T.....AC.A.AG.GCC.CCA.GGTG..A...AAC...CAG		
hEBV	...G.....T.C..T....C.....AC.ACG.GG.C...A.G.CAAAAG.T...C.GAC	(ENTFA>)	gtcaacggagtaga-
		(<CFSSB)	acraartcragntacttctaacaacttg
		(SHMDA>)	ycayatgga

FIG. 17B

KSHV	AAACACATTACTGACAGACGATGTTAACACCACAGTATTCTCCAACCAGTAGAGG
RFHV	...T..G..C..C..TC.G..T..C..A...AA..C.....T.....G..C..C..A..
bHV4	G....C.A..TA.....G..TTC..AA..T.AGT.T..TCC.....G.....G.CC..
shV1	...TT.T.ACTA.....T.GAAA.....AA..T...GA.T.AA.G..T.....T...
eHV2	C....C.ACTA.....G...GGTGG...GAG..C.CC.....A....G.CC..T...
mHV68	.C.GGTG.A.GTG.....T.GG..C..T.AA..T...GAA.A.A.GC..T..T..T...
hEBV	GCG.GTG.A.GTA...C.C....GA.....T...C..CAA...AA.G..CACC.G...
	-raayacnttacynga (ENTFA>)
KSHV	GCTTACGGATAACATTCAAAGGTACTTTAGCCAGCCGGTCATCTACGCGAACC GGCTG
RFHV	T..A..T..C....A....A.....A.....A..A..AC.G..T.T.....A...
bHV4	A..G..T..G...TAA.C.A....T..T..A..TC....A..T.A....T.....
shV1	T..A...G.GCA.T.AC...A..A.T....A..TAAAG.T.TT..T..T..T....
eHV2	T..C..CTC..G..TAACGC.C..TCAG..T..A..A.AGG.G....CACC...A.AAA
mHV68	T..A..A.GG..T..C.....A.....T.....CAC.C.T..TT.A.....T..T...
hEBV	C..GG.CA.CGGG.G.GCC.C...GCC.....A...AGC.....T.AC.CC.....G...
	tgnctrtrtaagttcccatgaaatcggtcggtca (<DNIQB)
KSHV	GTTTCCCGGCATATACAGAGTTAGG
RFHV	...C..A..T..C.....G...G...
bHV4A..G..T...T.....G..A
shV1	.C.ATGG..A.CT.....GAC.C.A
eHV2	CC.GTTGT.GTCT....C.ACA..A
mHV68	.A.G..T...T..T...TC.T...C.A
hEBV	...GATAT.G.CT.....ACA..A



FIG. 18

FIG. 19A*Capsid/Maturation/Transport Gene*

G G M F P I Q K M M V S E M I W P S I E
TGGGGCATGTTCCCATTCAAAAGATGATGGTATCAGAGATGATCTGGCCCAAGCATAGA 60

R K D W I E P N F N Q F Y S F E N Q D I
GCGAAGGACTGGATAGAGCCCAACTTCAACCAGTTCTATAGCTTTGAGAATCAAGACAT 120

N H L Q K R A W E Y I R E L V L S V S L
AAACCATCTGCAAAAGAGAGCTTGGGAATATATCAGAGAGCTGGTATTATCGGTTTCTCT 180

N N R T W E R E L K I L L T P Q G S P G
GAACAACAGAACTTGGGAGAGGGAGCTAAAATACTTCTCAGCCTCAGGGCTCACCGGG 240

F E E P K P A G L T T G L Y L T F E I S
GTTTGAGGAACCGAAACCCGAGGACTCACACGGGGCTGTACCTAACATTGAGATATC 300

A P L V L V D K K Y G W I F K D L Y A L
TGCGCCCTTGGTGTGGATAAAAAATATGGCTGGATATTTAAAGACCTGTACGCCCT 360

L Y H H L Q L S N H N D S Q V * *Glycoprotein B Gene*
M T P R S R L A T L
TCTGTACCACCACTGCAACTGAGCAACCAACAATGACTCCCAGGTCTAGATTGGCCACCC 420

G T V I L L V C F C A G A A H S R G D T
TGGGACTGTATCCTGTTGGTCTGCTTTTGGCAGCGCGGCGCACTCGAGGGGTGACA 480

FIG. 19B

F Q T S S P T P P G S S K A P T K P
CCTTTCAGACGTCAGTTCCCCACACCCCCAGGATCTTCCCTCTAAGGCCCCCACCAC
540

G E E A S G P K S V D F Y Q F R V C S A
CTGGTGAGGAAGCATCTGGTCCCTAAGAGTGTGGACTTTTACCAGTTCAGAGTGTGTAGTG
600

S I T G E L F R F N L E Q T C P D T K D
CATCGATCACCGGGAGCTTTTTCGGTTCAACCTGGAGCAGACGTGCCCAGACACCAAAG
660

K Y H Q E G I L L V Y K K N I V P H I F
ACAAGTACCACCAAGAAGGAATTTTACTGGTGTAACAAAACATAGTGCCTCATATCT
720

K V R R Y R K I A T S V T V Y R G L T E
TTAAGTGCGGCTATAGGAAAATTGCCACCTCTGTACCGTCTACAGGGCTTGACAG
780

S A I T N K Y E L P R P V P L Y E I S H
AGTCCGCCATCACCAACAAGTATGAACCTCCGAGACCCGTGCCACTCTATGAGATAAGCC
840

M D S T Y Q C F S S M K V N V N G V E N
ACATGGACAGCACCTATCAGTGCTTTAGTTCATGAAGTAAATGTCAACGGGTAGAAA
900

FIG. 19C

T F T D R D D V N T T V F L Q P V E G L 960
ACACATTTACTGACAGACGATGTTAACACACAGTATTCTCCAACCACTAGAGGGC
T D N I Q R Y F S Q P V I Y A E P G W F 1020
TTACGGATAACATTCAAGTACTTTAGCCAGCCGGTCACTACGCCGAACCCGGCTGGT
P G I Y R V R T T V N C E I V D M I A R 1080
TTCCCGGCATATACAGAGTTAGGACCACTGTCAATTGCGAGATAGTGACATGATAGCCA
> C
S A E P Y N Y F V T S L G D T V E V S P 1140
GGTCTGCTGAACCATACAATTACTTTGTCACTGCTCACTGGTGACACGGTGGAAGTCTCCC
F C Y N E S S C S T T P S N K N G L S V 1200
CTTTTGTATAAACGAATCCTCATGCAGCACAAACCCAGCAACAAAATGGCCTTAGCG
Q V V L N H T V V T Y S D R G T S P T P 1260
TCCAAGTAGTTCTCAACCACACTGTGGTCACGTACTCTGACAGAGGAACCACTCCCACTC
Q N R I F V E T G A Y T L S W A S E S K 1320
CCCAAAACAGGATCTTTGTGGAACGGGAGCGGTACACGCTTTCGTGGCCCTCCGAGAGCA
T T A V C P L A L W K T F P R S I Q T T 1380
AGACCACGGCGTGTCCGCTGGCACTGTGGAACCTTCCCGCGCTCCATCCAGACTA

FIG. 19D

H E D S F H F V A N E I T A T F T A P L
CCCACGAGACAGCTTCCACTTTGTGGCCAACGAGATCAGGCCACCTTCACGGTCTCCTC
1440

T P V A N F T D T Y S C L T S D I N T T
TAACGCCAGTGGCCAACTTTACCGACACGTACTCTTGTCTGACCTCGGATATCAACACCA
1550

L N A S K A K L A S T H V P N G T V Q Y
CGCTTAACGCCAGCAAGGCCAAACTGGCGAGCACTCACGTCCCTAACGGACGGTCCAGT
1560

F H T T G G L Y L V W Q P M S A I N L T
ACTTCCACACAACAGCGGACTCTATTTGGTCTGGCAGCCCATGTCCGCGATTAACTTGA
1620

H A Q G D S G N P T S S P P P S A S P M
CTCACGCTCAGGGCGACAGCGGGAACCCACGTATCGCCGCCCCCTCCGCATCCCCCA
1680

T T S A S R R K R S A S T A A A G G G
TGACCACTCTGCCAGCCGAGAAAGACGGTCAGCCAGTACCGTGTCTGCCGGCGCGG
1740

G S T D N L S Y T Q L Q F A Y D K L R D
GGGGTCCACGGACAACCTGTCTTACACGCAGCTGCAGTTTGCCTACGACAAACTGCGGG
1800

G I N Q V L E E L S R A W C R E Q V R D
ATGGCATTAATCAGGTGTAGAAGAACTCTCCAGGGCATGGTGTCTCGCAGCAGGTACAGG
1860

FIG. 19E

N L M W Y E L S K I N P T S V M T A I Y 1920
ACAACCTAATGTGTACGAGCTCAGTAAATCAACCCACCAGCGTTATGACAGCCATCT
G R P V S A K F V G D A I S V T E C I N 1980
ACGGTCGACCTGTATCCGCCAAGTTCGTAGGAGACGCCATTTCCTGACCGAGTGCATTA
V D Q S S V N I H K S L R T N S K D V C 2040
ACGTGGACCAGAGCTCCGTAAACATCCACAAGAGCCTCAGAACCAATAGTAAGGACGTGT
Y A R P L V T F K F L N S S N L F T G Q 2100
GTTACGCGGCCCTGTGTGACGTTTAAGTTTTTGAACAGTTCCAACCTATTCAACGGCC
L G A R N E I I L T N N Q V E T C K D T 2160
AGCTGGCGCGCAATGAGATAATACTGACCAACAACCAAGGTGGAACCTGCAAAAGACA
C E H Y F I T R N E T L V Y K D Y A Y L 2220
CCTGCGAACACTACTTCATCACCCGCAACGAGACTCTGGTGTATAAGGACTACGCGTACC
R T I N T T D I S T L N T F I A L N L S 2280
TGCGCACTATAAACACCACTGACATATCCACCTGAAGAGTTTTATCGCCCTGAATCTAT
F I Q N I D F K A I E L Y S S A E K R L 2340
CCTTTATTCAAACATAGACTTCAAGGCCATCGAGCTGTACAGCAGTGCAGAGAAACGAC
A S S V F D L E T M F R E Y N Y Y T H R 2400
TCGCGAGTAGCGTGTGTGACCTGGAGACGATGTTTCAGGGAGTACAACACTACACACATC

FIG. 19F

L A G L R E D L D N T I D M N K E R F V 2460
GTCTCGCGGTTTGGCGAGGATCTGGACAACACCATAGATATGAACAAGGAGCGCTTCG
R D L S E I V A D L G G I G K T V V N V 2520
TAAGGACTTGTCTCGAGATAGTGGCGGACCTGGTGGCATCGGAAAACGGTGGTGAACG
>T
A S S V V T L C G S L V T G F I N F I K 2580
TGGCCAGCAGCGTGGTCACTCTATGTGGCTCATTTGGTTACCGGATTCATAAATTTATTA
H P L G G M L M I I I V I A I I L I I F 2640
AACACCCCCTAGGTGGCATGCTGATCATTCATTATCGTTATAGCAATCATCCTGATCATT
M L S R R T N T I A Q A P V K M I Y P D 2700
TTATGCTCAGTCGCCGCACCAATACCATAGCCCAGGCGCGGTGAAGATGATCTACCCCG
V D R R A P P S G G A P T R E E I K N I 2760
ACGTAGATCGAGGGCACCTCCTAGCGGCGGAGCCCCAACACGGGAGGAATCAAAAACA

FIG. 19G

L L G M H Q L Q Q E E R Q K A D D L K K
TCCTGCTGGGAATCGACCACTACAACAAGAGGAGAGCGGATGATCTGAAAA
2820
> T

S T P S V F Q R T A N G L R Q R L R G Y
AAAGTACACCCCTCGGTGTTTCAGCGTACCGCAACGGCCTTCGTACGCTCTGAGAGGAT
2880

K P L T Q S L D I S P E T G E *
ATAAACCTCTGACTCAATCGCTAGACATCAGTCCGGAACGGGGAGTGACAGTGGATTC
2940
> T

GAGGTATTGTTTGATGTAATTTAGGAACACGGCCCGCCTCTGAAGCACCATACAG
3000

DNA POLYMERASE GENE
M D
ACTGCAGTTATCAACCCTACTCGTTGCACACAGACACAATTACCGTCCGCAGATCATGG
3060

F F N P F I D P T R G G P R N T V R Q P
ATTTTTCATCCATTATCGACCCCACTCGCGAGGCCCGAGAAACACTGTGAGGCAAC
3120

T P S Q S P T V P S E T R V C R L I P A
CCACGCCGTCACAGTCGCCAATGTCCCCCTCGGAGACAAAGAGTATGCAGGCTTATACCGG
3180

FIG. 19H

C F Q T P G R P G V V A V D T T F P P T
CCTGTTTCCAAACCCCGGGGACCCGGCGTGGTTGCCGTGGACACCATTTCCACCCA 3240

Y F Q G P K R G E V F A G E T G S I W K
CCTACTTCCAGGGCCCCAAGCGGGAGAGTATTCGGGGGAGAGACTGGGTCTATCTGGA 3300

T R R G Q A R N A P M S H L I F H V Y D
AAACAAGGCGGACAGGCACGCAATGCTCCTATGTGCGCACCTCATATTTCCACGTATACG 3360

I V E T T Y T A D R C E D V P F S F Q T
ACATCGTGGAGACCCTACACGGCCGACCGCTGCGAGGACGTGCCATTTAGCTTCCAGA 3420

D I I P S G T V L K L L G R T L D G A S
CTGATATCATTTCCAGCGGCACCGTCTCAAGCTGCTCGGCAGAACACTAGATGGCGCCA 3480

V C V N V F R Q R C Y F Y T L A P Q G V
GTGTCGCGTGAACGTTTTCAGGCAGCGCTGCTACTTCTACACACTAGACCCAGGGGG 3540

N L T H V L Q Q A L Q A G F G R A S C G
TAAACCTGACCCACGTCTCCAGCAGGCCCTCCAGGCTGGCTTCGGTCGCGCATCCTGCG 3600

F S T
GCTTCTCCACCG 3612

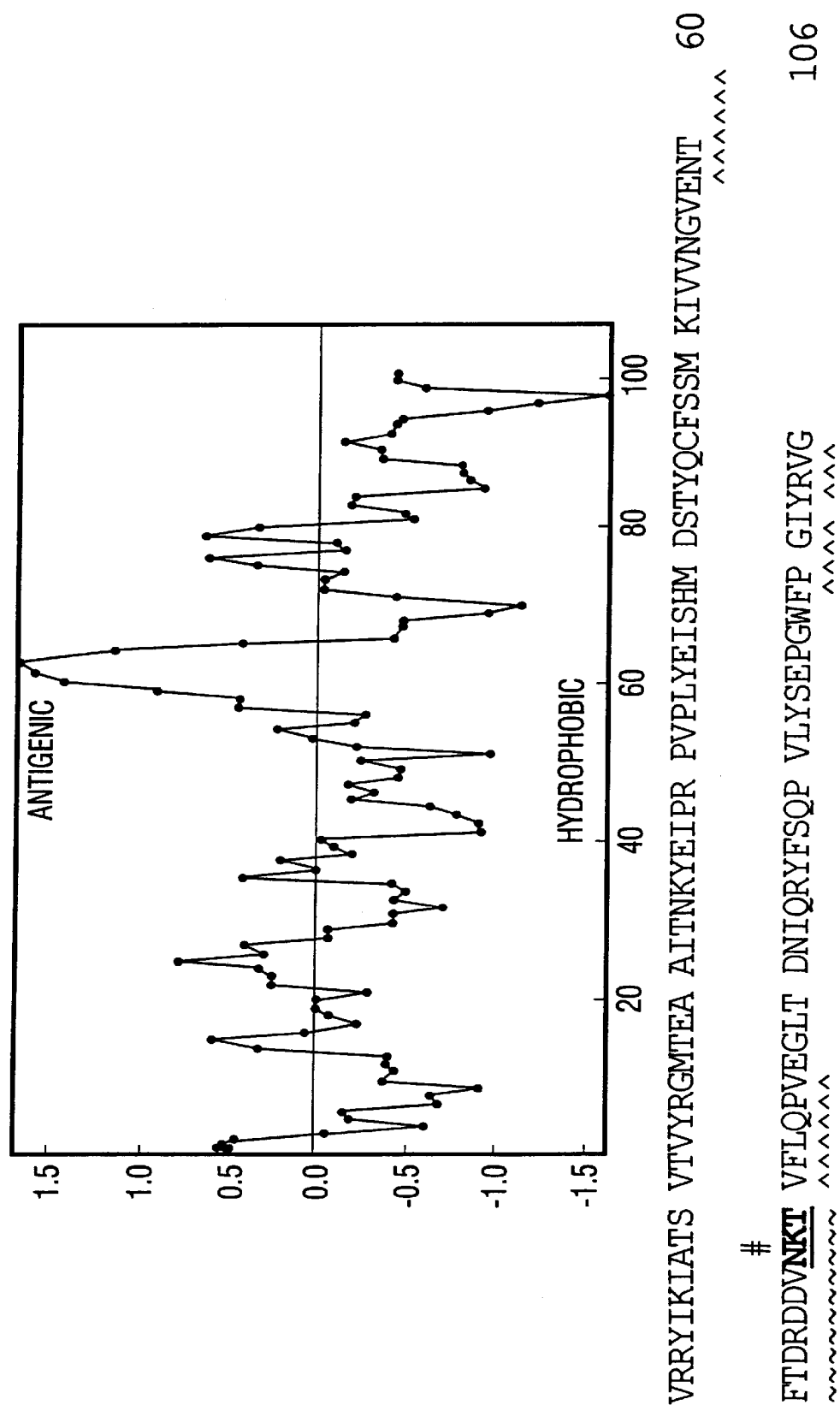


FIG. 20

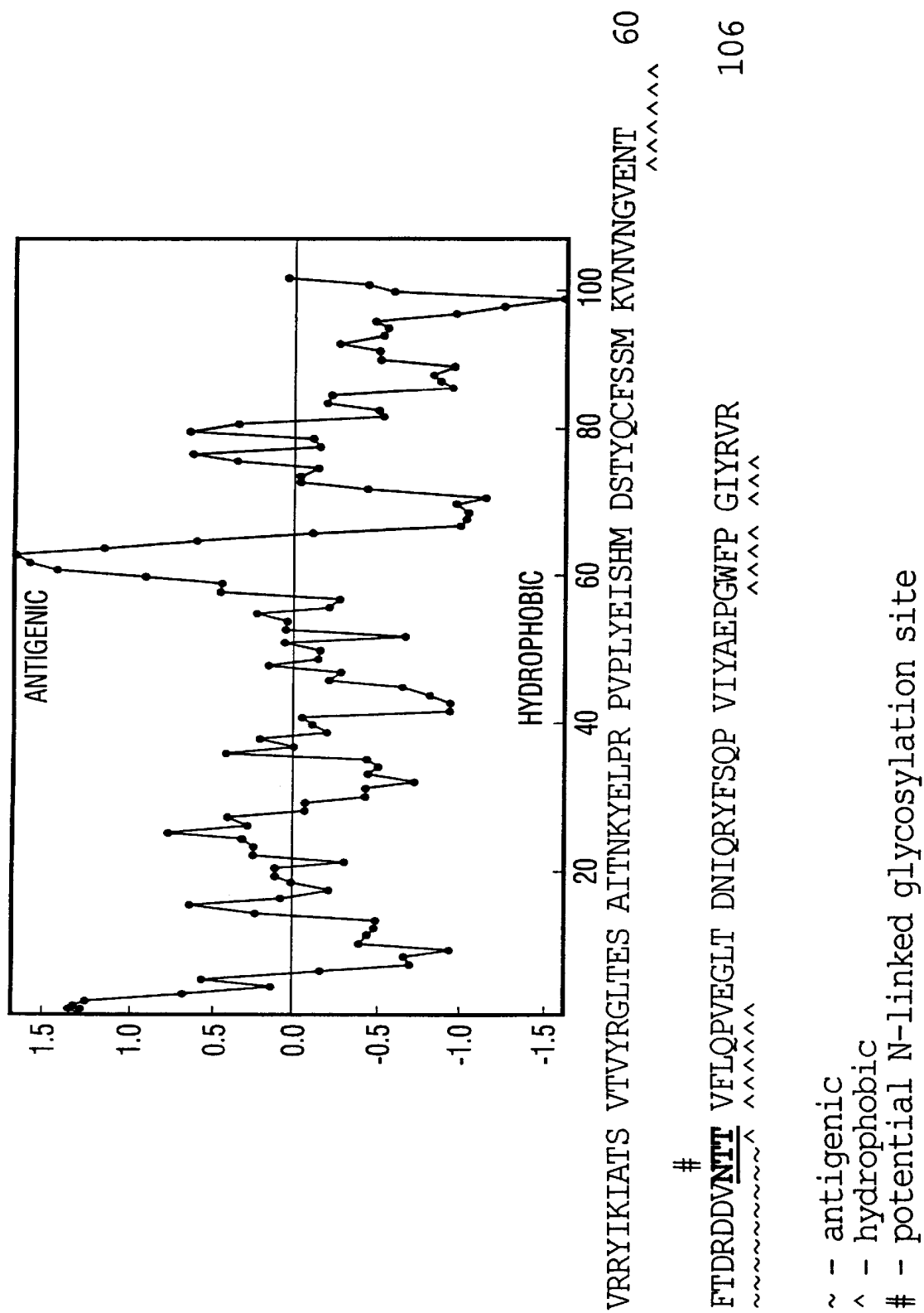


FIG. 21

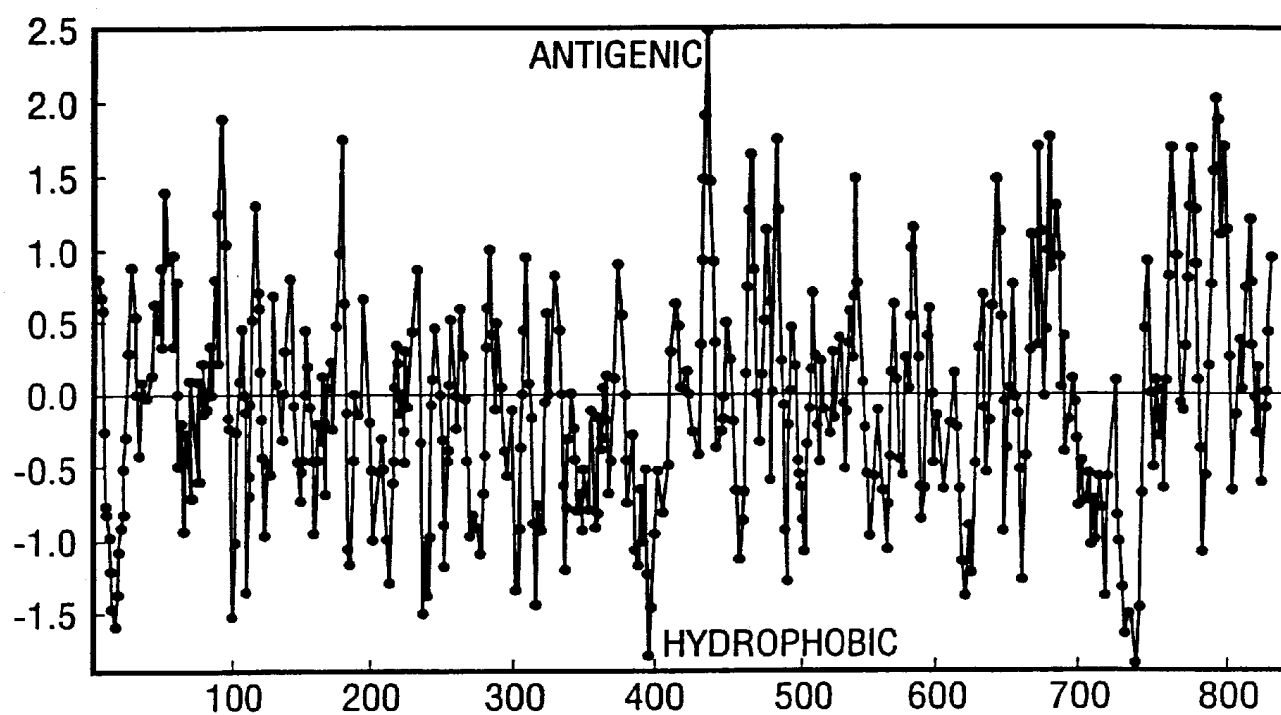
**FIG. 22**

FIG. 23

V Y K K N I V P N M F K V R R Y R K V A 60
GTGTACAAGAAGAACATCGTGCCTAACATGTTCAAGGTACGCAGGTACAGAAAAGTAGCA

T P V T L Y R G M T D A A I T N K Y E I 120
ACGCCTGTCACACTCTACCCGGGTATGACAGACGCAGCAATAACTAACAATATGAAATT

P R P V P L Y E I S H M D S T Y Q C F S 180
CCCAGACCCGTACCACTATACGAGATCAGTCACATGGACAGCACCTACCAGTGCTTTAGT

S M K I V V N G V E N T F T G R D D V N 240
TCCATGAAAATTGTAGTGAACGGAGTCGAAAACACACGTTCAACCGTCGGGATGACGTAAAC

K S V F L Q P V E G L T D N I K R Y F S 300
AAAAGCGTATTTCTCCAGCCAGTCGAAGGTCTAACTGACAACATAAAGAGATACTTTAGC

Q P V L Y S E P G W F P G I Y R V R T T 360
CAGCCAGTGTATATTCTGAACCCGGATGGTTTCCAGGTATCTACAGGGTTAGGACAACA

V N C E I V D M 380
GTTAATTGTGAGATTGTAGACATGTT

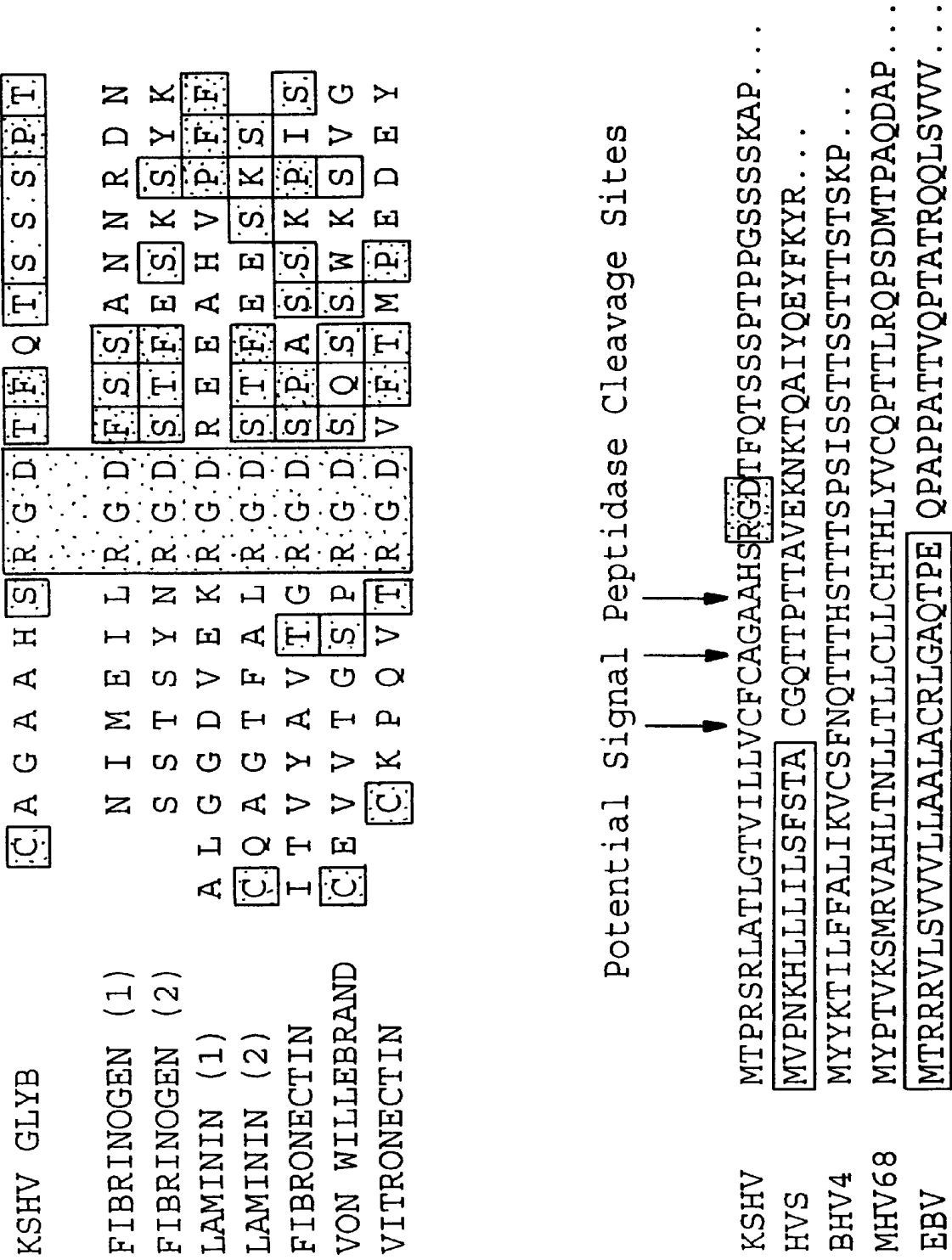


FIG. 24

GLYCOPROTEIN B OF THE RFHV/KSHV SUBFAMILY OF HERPES VIRUSES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of International Application PCT/US96/15702 designating the U.S., which was filed on Sep. 26, 1996; for which the priority application is U.S. provisional patent application Ser. No. 60/004,297, filed Sep. 26, 1995. This application is also a continuation-in-part of U.S. non-provisional application Ser. No. 08/720,229, filed Sep. 26, 1996, pending; for which the priority application is U.S. provisional patent application Ser. No. 60/004,297, filed Sep. 26, 1995, now abandoned. This application claims priority benefit of all the above-referenced applications, which are hereby incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

The present invention relates generally to the field of virology, particularly viruses of the herpes family. More specifically, it relates to the identification and characterization of herpes virus Glycoprotein B molecules which are associated with fibroproliferative and neoplastic conditions in primates, including humans.

BACKGROUND

Kaposi's Sarcoma is a disfiguring and potentially fatal form of hemorrhagic sarcoma. It is characterized by multiple vascular tumors that appear on the skin as darkly colored plaques or nodules. At the histological level, it is characterized by proliferation of relatively uniform spindle-shaped cells, forming fascicles and vascular slits. There is often evidence of plasma cells, T cells and monocytes in the inflammatory infiltrate. Death may ultimately ensue due to bleeding from gastrointestinal lesions or from an associated lymphoma. (See generally Martin et al., Finesmith et al.)

Once a relatively obscure disease, it has leapt to public attention due to its association with AIDS. As many as 20% of certain AIDS-affected populations acquire Kaposi's during the course of the disease. Kaposi's Sarcoma occurs in other conditions associated with immunodeficiency, including kidney dialysis and therapeutic immunosuppression. However, the epidemiology of the disease has suggested that immunodeficiency is not the only causative factor. In particular, the high degree of association of Kaposi's with certain sexual practices suggests the involvement of an etiologic agent which is not the human immunodeficiency virus (Berel et al.).

A herpes-virus-like DNA sequence has been identified in tissue samples from Kaposi's lesions obtained from AIDS patients (Chang et al., confirmed by Ambroziuk et al.). The sequence was obtained by representational difference analysis (Lisitsyn et al.), in which DNA from affected and unaffected tissue were amplified using unrelated priming oligonucleotides, and then hybridized together to highlight differences between the cells. The sequence was partly identical to known sequences of the Epstein Barr Virus and herpesvirus saimiri. It coded for capsid and tegument proteins, two structural components sequestered in the viral interior. In a survey of tissues from various sources, the sequence was found in 95% of Kaposi's sarcoma lesions, regardless of the patients' HIV status (Moore et al. 1995a). 21% of uninvolved tissue from the same patients was positive, while 5% of samples from a control population was

positive. There was approximately 0.5% sequence variation between samples.

The same sequence has been detected in body cavity lymphoma, a lymphomatous effusion with B-cell features, occurring uniquely in AIDS patients (Cesarman et al.). The copy number was higher in body cavity lymphoma, compared with Kaposi's Sarcoma. Other AIDS-associated lymphomas were negative. The sequence has also been found in peripheral blood mononuclear cells of patients with Castleman's disease (Dupin et al.). This is a condition characterized by morphologic features of angiofollicular hyperplasia, and associated with fever, adenopathy, and splenomegaly. The putative virus from which the sequence is derived has become known as Kaposi's Sarcoma associated Herpes Virus (KSHV).

Using PCR in situ hybridization, Boshoff et al. have detected KSHV polynucleotide sequences in the cell types thought to represent neoplastic cells in Kaposi's sarcoma. Serological evidence supports an important role for KSHV in the etiology of Kaposi's sarcoma (O'Leary). Kedes et al. developed an immunofluorescence serological assay that detects antibody to a latency-associated nuclear antigen in B cells latently infected with KSHV, and found that KSHV seropositivity is high in patients with Kaposi's sarcoma. Gao et al. found that of 40 patients with Kaposi's sarcoma, 32 were positive for antibodies against KSHV antigens by an immunoblot assay, as compared with only 7 of 40 homosexual men without Kaposi's sarcoma immediately before the onset of AIDS. Miller et al. prepared KSHV antigens from a body cavity lymphoma cell line containing the genomes of both KSHV and Epstein-Barr virus. Antibodies to one antigen, designated p40, were identified in 32 of 48 HIV-1 infected patients with Kaposi's sarcoma, as compared with only 7 of 54 HIV-1 infected patients without Kaposi's sarcoma.

Zhong et al. analyzed the expression of KSHV sequences in affected tissue at the messenger RNA level. Two small transcripts were found that represent the bulk of the virus specific RNA transcribed from the KSHV genome. One transcript was predicted to encode a small membrane protein; the other is an unusual poly-A RNA that accumulates in the nucleus and may have no protein encoding sequence. Messenger RNA was analyzed by cloning a plurality of overlapping KSHV genomic fragments that spanned the ~120 kb KSHV genome from a lambda library of genomic DNA. The clones were used as probes for Northern analysis, but their sequences were not obtained or disclosed.

Moore et al. have partially characterized a KSHV genome fragment obtained from a body-cavity lymphoma. A 20.7 kb region of the genome was reportedly sequenced, although the sequence was not disclosed. 17 partial or complete open reading frames were present in this fragment, all except one having sequence and positional homology to other known gamma herpes virus genes, including the capsid maturation gene and the thymidine kinase gene. Phylogenetic analysis showed that KSHV was more closely related to equine herpes virus 2 and Saimiri virus than to Epstein Barr virus. The 20.7 kb region did not contain sequences encoding either Glycoprotein B or DNA polymerase.

The herpes virus family as a whole comprises a number of multi-enveloped viruses about 100 nm in size, and capable of infecting vertebrates. (For general reviews, see, e.g., Emery et al., Fields et al.). The double-stranded DNA genome is unusually large—from about 88 to about 229 kilobases in length. It may produce over 50 different transcripts at various stages in the life cycle of the virus. A

number of glycoproteins are expressed at the viral surface, and play a role in recognition of a target cell by the virus, and penetration of the virus into the cell. These surface proteins are relatively more variant between species, compared with internal viral components (Karlin et al.). The same surface proteins are also present on defective viral particles produced by cells harboring the virus. One such non-infectious form is the L-particle, which comprises a tegument and a viral envelope, but lacks the nucleocapsid.

The herpes virus family has been divided into several subfamilies. Assignments to each of the categories were originally based on biologic properties, and are being refined as genomic sequence data emerges. The alpha subfamily comprises viruses that have a broad host range, a short replicative cycle, and an affinity for the sensory ganglia. They include the human simplex virus and the Varicella-zoster virus. The beta subfamily comprises viruses that have a restricted host range, and include Cytomegalovirus and human Herpes Virus 6. The gamma subfamily comprises viruses that are generally lymphotropic. The DNA is marked by a segment of about 110 kilobases with a low GC content, flanked by multiple tandem repeats of high GC content. The gamma subfamily includes Epstein Barr Virus (EBV), herpes virus saimiri, equine Herpes Virus 2 and 5, and bovine Herpes Virus 4.

Herpes viruses are associated with conditions that have a complex clinical course. A feature of many herpes viruses is the ability to go into a latent state within the host for an extended period of time. Viruses of the alpha subfamily maintain latent forms in the sensory and autonomic ganglia, whereas those of the gamma subfamily maintain latent forms, for example, in cells of the lymphocyte lineage. Latency is associated with the transcription of certain viral genes, and may persist for decades until conditions are optimal for the virus to resume active replication. Such conditions may include an immunodeficiency. In addition, some herpes viruses of the gamma subfamily have the ability to genetically transform the cells they infect. For example, EBV is associated with B cell lymphomas, oral hairy leukoplakia, lymphoid interstitial pneumonitis, and nasopharyngeal carcinoma.

A number of other conditions occur in humans and other vertebrates that involve fibroproliferation and the generation of pre-neoplastic cells. Examples occurring in humans are retroperitoneal fibrosis, nodular fibromatosis, pseudosarcomatous fibromatosis, and sclerosing mesenteritis. Another condition known as Enzootic Retroperitoneal Fibromatosis (RF) has been observed in a colony of macaque monkeys at the University of Washington Regional Primate Research Center (Giddens et al.). Late stages of the disease are characterized by proliferating fibrous tissue around the mesentery and the dorsal part of the peritoneal cavity, with extension into the inguinal canal, through the diaphragm, and into the abdominal wall. Once clinically apparent, the disease is invariably fatal within 1–2 months. The condition has been associated with simian immunodeficiency (SAIDS) due to a type D simian retrovirus, SRV-2 (Tsai et al.). However, other colonies do not show the same frequency of RF amongst monkeys affected with SAIDS, and the frequency of RF at Washington has been declining in recent years.

The study of such conditions in non-human primates is important not only as a model for human conditions, but also because one primate species may act as a reservoir of viruses that affect another species. For example, the herpes virus saimiri appears to cause no disease in its natural host, the squirrel monkey (*Saimiri sciureus*), but it causes polyclonal

T-cell lymphomas and acute leukemias in other primates, particularly owl monkeys.

There is a need to develop reagents and methods for use in the detection and treatment of herpes virus infections. The etiological linkage between KSHV and Kaposi's sarcoma, confirmed by the serological evidence, indicates the importance of this need.

For example, there is a need to develop reagents and methods which can be used in the diagnosis and assessment of Kaposi's sarcoma, and similar conditions. Being able to detect the etiologic agent in a new patient may assist in differential diagnosis; being able to assess the level of the agent in an ongoing condition may assist in clinical management. Desirable markers include those that provide a very sensitive indication of the presence of both active and latent forms viral infection, analogous to the HBsAg of Hepatitis B. Desirable markers also include those that are immunogenic, and can be used to assess immunological exposure to the viral agent as manifest in the antibody response. Glycoprotein antigens from the viral envelope are particularly suitable as markers with these characteristics. They may be expressed at high abundance near the surface not only of replicative forms of the virus, but also on L-particles produced by virally infected cells.

Second, there is a need to develop reagents and methods that can be used for treatment of viral infection—both prophylactically, and following a viral challenge. Such reagents include vaccines that confer a level of immunity against the virus. Passive vaccines, such as those comprising an anti-virus antibody, may be used to provide immediate protection or prevent cell penetration and replication of the virus in a recently exposed individual. Active vaccines, such as those comprising an immunogenic viral component, may be used to elicit an active and ongoing immune response in an individual. Antibody elicited by an active vaccine may help protect an individual against a subsequent challenge by live virus. Cytotoxic T cells elicited by an active vaccine may help eradicate a concurrent infection by eliminating host cells involved in viral replication. Suitable targets for a protective immune response, particularly antibody, are protein antigens exposed on the surface of viral particles, and those implicated in fusion of the virus with target cells.

Third, there is a need to develop reagents and methods which can be used in the development of new pharmaceuticals for Kaposi's sarcoma, and similar conditions. The current treatment for Kaposi's is radiation in combination with traditional chemotherapy, such as vincristine (Northfelt, Mitsuyasu). While lesions respond to these modalities, the response is temporary, and the downward clinical course generally resumes. Even experimental therapies, such as treatment with cytokines, are directed at the symptoms of the disease rather than the cause. Drug screening and rational drug design based upon the etiologic agent can be directed towards the long-felt need for a clinical regimen with long-term efficacy. Suitable targets for such pharmaceuticals are viral components involved in recognition and penetration of host cells. These include glycoprotein components of the viral envelope.

Fourth, there is a need to develop reagents and methods which can be used to identify new viral agents that may be associated with other fibroproliferative conditions. The representational difference analysis technique used by Chang et al. is arduously complex, and probably not appropriate as a general screening test. More desirable are a set of oligonucleotide probes, peptides, and antibodies to be used as reagents in more routine assays for surveying a variety of

tissue samples suspected of containing a related etiologic agent. The reagents should be sufficiently specific to avoid identifying unrelated viruses and endogenous components of the host, and may be sufficiently cross-reactive to identify related but previously undescribed viral pathogens.

SUMMARY OF THE INVENTION

It is an objective of this invention to provide isolated polynucleotides, polypeptides, and antibodies derived from or reactive with the products of novel genes encoding Glycoprotein B molecules of the RFHV/KSHV subfamily of herpes viruses. Two members of the family are Retroperitoneal Fibromatosis associated Herpes Virus (RFHV) and Kaposi's Sarcoma associated Herpes Virus (KSHV). These materials and related methods can be used in the diagnosis and treatment of herpes virus infection in primates, including humans. Isolated or recombinant Glycoprotein B fragments or polynucleotides encoding them may be used as components of an active herpes vaccine, while antibodies specific for Glycoprotein B may be used as components of a passive vaccine.

Accordingly, one of the embodiments of the invention is an isolated polynucleotide with a region encoding a Glycoprotein B of a herpes virus of the RFHV/KSHV subfamily, the polynucleotide comprising a sequence of 319 nucleotides at least 65% identical to nucleotides 36 to 354 of SEQ. ID NO:1 or SEQ. ID NO:3, which are 319 nucleotide fragments encoding Glycoprotein B from RFHV and KSHV, respectively. Also embodied is an isolated polynucleotide with a region encoding a Glycoprotein B, the polynucleotide comprising a sequence selected from the group consisting of: a sequence of 35 nucleotides at least 74% identical to oligonucleotide SHMDA (SEQ. ID NO:41); a sequence of 30 nucleotides at least 73% identical to oligonucleotide CFSSB (SEQ. ID NO:43); a sequence of 29 nucleotides at least 72% identical to oligonucleotide ENTFA (SEQ. ID NO:45); and a sequence of 35 nucleotides at least 80% identical to oligonucleotide DNIQB (SEQ. ID NO:46).

Another embodiment of the invention is an isolated polynucleotide comprising a fragment of at least 21, preferably 35, more preferably 50, still more preferably 75, and even more preferably 100 consecutive nucleotides of the Glycoprotein B encoding region of the polynucleotide of the preceding embodiments. The polynucleotide is preferably from a virus capable of infecting primates. Included are Glycoprotein B encoding polynucleotide fragments from RFHV and KSHV. Another embodiment of the invention is an isolated polynucleotide comprising a linear sequence of at least about 21 nucleotides identical to a the Glycoprotein B encoding sequence between nucleotides 36 to 354 inclusive of SEQ. ID NO:1, SEQ. ID NO:3, or SEQ. ID NO:92, or anywhere within SEQ. ID NO:96, but not in SEQ. ID NO:98.

A further embodiment of this invention is an isolated polypeptide encoded by any of the previous embodiments. Also embodied is an isolated polypeptide, comprising a linear sequence of at least 17 amino acids essentially identical to the Glycoprotein B protein sequence shown in SEQ. ID NO:2, SEQ. ID NO:4, or SEQ. ID NO:97, or anywhere within SEQ. ID NO:94 (KSHV), but not in SEQ. ID NO:99. This includes fusion polypeptides, immunogenic polypeptides, and polypeptides occurring in glycosylated and unglycosylated form. Some preferred antigen peptides are listed in SEQ. ID NOS:67-76. Also embodied are isolated and non-naturally occurring polynucleotides encoding any of the aforementioned polypeptides, along with

cloning vectors, expression vectors and transfected host cells derived therefrom. Further embodiments are method for producing polynucleotides or polypeptides of this invention, comprising replicating vectors of the invention or expressing polynucleotides in suitable host cells.

Yet another embodiment of this invention is a monoclonal or isolated polyclonal antibody specific for a Glycoprotein B polypeptide embodied in this invention, or a Glycoprotein B encoded in the encoding region of a polynucleotide embodied in this invention. The antibodies are specific for members of the RFHV/KSHV subfamily, and do not cross-react with more distantly related Glycoprotein B sequences, particularly SEQ. ID NOS:30-41.

Still another embodiment of this invention is a vaccine comprising a polypeptide of this invention in a pharmaceutically compatible excipient, and optionally also comprising an adjuvant. In certain embodiments, the polypeptide of the vaccine comprises an RGD sequence. Another embodiment of this invention is a vaccine comprising a polynucleotide of this invention, which may be in the form of a live virus or viral expression vector. Another embodiment of this invention is a vaccine comprising an antibody of this invention in a pharmaceutically compatible excipient. Other embodiments are methods for treating a herpes virus infection, either prophylactically or during an ongoing infection, comprising administering one of the aforementioned embodiments.

Also embodied in this invention are methods of inhibiting attachment of a herpes virus to a cell, or preventing infection or pathology due to a member of the RFHV/KSHV virus subfamily, comprising contacting the cell or introducing into the environment a polypeptide according to this invention comprising an RGD sequence.

Further embodiments of this invention are oligonucleotides specific for Glycoprotein B encoding sequences of the gamma herpes subfamily, the RFHV/KSHV subfamily, RFHV, and KSHV, especially those listed in SEQ. ID NOS:24-63. Also embodied are methods for obtaining an amplified copy of a polynucleotide encoding a Glycoprotein B, comprising contacting the polynucleotide with one or more of the aforementioned oligonucleotides. The polynucleotide to be amplified may be taken from an individual affected with a disease featuring fibroblast proliferation and collagen deposition, including but not limited to Retroperitoneal Fibromatosis or Kaposi's Sarcoma, or a malignancy of the lymphocyte lineage.

Additional embodiments of this invention are methods for detecting viral DNA or RNA in a sample. One method comprises the steps of contacting the DNA or RNA in the sample with a probe comprising a polynucleotide or oligonucleotide of this invention under conditions that would permit the probe to form a stable duplex with a polynucleotide having the sequence shown in SEQ. ID NO:1 or SEQ. ID NO:3, or both, but not with a polynucleotide having a sequence of herpes viruses outside the RFHV/KSHV subfamily, particularly SEQ. ID NOS:5-13, and detecting the presence of any duplex formed thereby. The conditions referred to are a single set of reaction parameters, such as incubation time, temperature, solute concentrations, and washing steps, that would permit the polynucleotide to form a stable duplex if alternatively contacted with a polynucleotide with SEQ. ID NO:1, or with a polynucleotide with SEQ. ID NO:3, or with both, but not with a polynucleotide of any of SEQ ID NO:5-13. Another method comprises the steps of amplifying the DNA or RNA in the sample using an oligonucleotide of this invention as a primer in the ampli-

fication reaction, and detecting the presence of any amplified copies. Also embodied are isolated polynucleotides identified by the aforementioned methods, as may be present in the genome of a naturally occurring virus or affected tissue.

Further embodiments of this invention are diagnostic kits for detecting components related to herpes virus infection in a biological sample, such as may be obtained from an individual suspected of harboring such an infection, comprising a polynucleotide, oligonucleotide, polypeptide, or antibody of this invention in suitable packaging. Also embodied are methods of detecting infection of an individual, comprising applying the reagents, methods, or kits of this invention on biological samples obtained from the individual.

Still other embodiments of this invention are therapeutic compounds and compositions for use in treatment of an individual for infection by a gamma herpes virus. Included are therapeutic agents that comprise polynucleotides and vectors of this invention for the purpose of gene therapy. Also included are pharmaceutical compounds identified by contacting a polypeptide embodied in this invention with the compound and determining whether a biochemical function of the polypeptide is altered. Also included are pharmaceutical compounds obtained from rational drug design, based on structural and biochemical features of a Glycoprotein B molecule.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a listing of polynucleotide sequences amplified from a Glycoprotein B encoding region of RFHV and KSHV. The 319-base polynucleotide segment between residues 36 to 354 is underlined, and represents the respective viral gene segment between the primers used to amplify it. Aligned with the polynucleotide sequences are oligonucleotides that may be used as hybridization probes or PCR primers. Type 1 oligonucleotides comprise a gamma herpes consensus sequence, and can be used to amplify a Glycoprotein B gene segment of a gamma herpes virus. Examples shown are NIVPA and TVNCB. Type 2 oligonucleotides comprise a consensus sequence from the RFHV/KSHV subfamily, and can be used to amplify Glycoprotein B gene segment of a virus belonging to the subfamily. Examples shown are SHMDA, CFSSB, ENTFA and DNIQB. The other oligonucleotides shown are Type 3 oligonucleotides. These comprise sequences taken directly from the RFHV or KSHV sequence, and are specific for sequences from the respective virus. Oligonucleotides that initiate amplification in the direction of the coding sequence (with designations ending in "A") are listed 5'→3'. Oligonucleotides that initiate amplification in the direction opposite to that of the coding sequence (with designations ending in "B") are listed 3'→5'. Also shown are the polypeptides encoded by the RFHV and KSHV polynucleotide sequences. The asparagine encoded by nucleotides 238–240 in both sequences is a potential N-linked glycosylation site conserved with other herpes viruses.

FIG. 2 is a map of the Glycoprotein B encoding DNA sequence believed to be contained in the KSHV genome, and other members of the RFHV/KSHV subfamily. Shown are the approximate location of the KSHV Glycoprotein B sequence described herein. Also shown are the putative conserved segments that represent hybridization sites for Type 1 consensus/degenerate oligonucleotides useful in probing and amplifying Glycoprotein B sequences from gamma herpes viruses.

FIGS. 3A–3D are listings of some previously known herpes virus Glycoprotein B protein sequences, aligned with

the complete KSHV Glycoprotein B protein sequence and fragments of RFHV1 and RFHV2. Boxed regions indicate the putative pre-processing signal sequence and the transmembrane domain. Cysteine residues are underlined. Residues that are highly conserved amongst herpes virus Glycoprotein B sequences are underscored with an asterisk (*). Cysteines appearing uniquely in the KSHV Glycoprotein B are underscored with a bullet (●).

FIG. 4 is a listing of previously known Glycoprotein B polynucleotide sequences of gamma herpes viruses, showing a conserved region, and the Type 1 oligonucleotide FRFDA designed therefrom.

FIG. 5 is a listing of previously known Glycoprotein B polynucleotide sequences of gamma herpes viruses, showing a conserved region, and the Type 1 oligonucleotides NIVPA and NIVPASQ designed therefrom.

FIG. 6 is a listing of previously known Glycoprotein B polynucleotide sequences of gamma herpes viruses, showing a conserved region, and the Type 1 oligonucleotides TVNCA, TVNCB and TVNCBSQ designed therefrom.

FIG. 7 is a listing of previously known Glycoprotein B polynucleotide sequences of gamma herpes viruses, showing a conserved region, and the Type 1 oligonucleotide FAYDA designed therefrom.

FIG. 8 is a listing of previously known Glycoprotein B polynucleotide sequences of gamma herpes viruses, showing a conserved region, and the Type 1 oligonucleotides IYGKA and IYGKASQ designed therefrom.

FIG. 9 is a listing of previously known Glycoprotein B polynucleotide sequences of gamma herpes viruses, showing a conserved region, and the Type 1 oligonucleotides CYSRA and CYSRASQ designed therefrom.

FIG. 10 is a listing of previously known Glycoprotein B polynucleotide sequences of gamma herpes viruses, showing a conserved region, and the Type 1 oligonucleotides NIDFB and NMDFBSQ designed therefrom.

FIG. 11 is a listing of previously known Glycoprotein B polynucleotide sequences of gamma herpes viruses, showing a conserved region, and the Type 1 oligonucleotides FREYA, FREYB and NVFDA designed therefrom.

FIG. 12 is a listing of previously known Glycoprotein B polynucleotide sequences of gamma herpes viruses, showing a conserved region, and the Type 1 oligonucleotide GGMA designed therefrom.

FIGS. 13A and 13B are listings of a portion of the Glycoprotein B polynucleotide sequence from RFHV and KSHV, aligned with previously known gamma herpes Glycoprotein B polynucleotide sequences. Each shared residue is indicated as a period.

FIG. 14 is a comparison listing of the polypeptide sequences of Glycoprotein B from various gamma herpes viruses, encoded between the hybridization sites of NIVPA and TVNCB in the polynucleotide sequences. The Class II sequence fragments shown underlined are predicted to be RFHV/KSHV cross-reactive antigen peptides. The Class III sequences shown in lower case are predicted to be RFHV or KSHV virus-specific peptides.

FIG. 15 is an alignment of the polypeptide sequences of Glycoprotein B over a broader spectrum of herpes viruses in the gamma, beta, and alpha subfamilies.

FIG. 16 is a relationship map of Glycoprotein B, based on the polypeptide sequences shown in FIG. 15.

FIGS. 17A and 17B are listings of exemplary Type 2 (subfamily-specific) oligonucleotides, aligned with the nucleotide sequences from which they were derived.

FIG. 18 is an approximate map of Glycoprotein B and DNA polymerase encoding regions as they appear in the KSHV genome, showing the hybridization position of oligonucleotide primers.

FIGS. 19A–19D of a KSHV DNA sequence obtained by amplifying fragments upstream and downstream from the sequence in FIG. 1. An open reading frame is shown for the complete KSHV Glycoprotein B sequence, flanked by open reading frames for the capsid maturation gene and DNA polymerase. Underlined in the nucleotide sequence is a putative Glycoprotein B promoter

FIG. 20 is a Hopp-Woods antigenicity plot for the 106 nucleotide Glycoprotein B polypeptide fragment of RFHV encoded between NIVPA and TVNCB. Indicated below are spans of hydrophobic and antigenic residues in the sequence.

FIG. 21 is a Hopp-Woods antigenicity plot for the 106 nucleotide Glycoprotein B polypeptide fragment of KSHV encoded between NIVPA and TVNCB.

FIG. 22 is a Hopp-Woods antigenicity plot for the complete Glycoprotein B from KSHV.

FIG. 23 is a listing of DNA and protein sequences for a Glycoprotein B fragment of a third member of the RFHV/KSHV subfamily, designated RFHV2. The 319-base polynucleotide segment between residues 36 to 354 is underlined, and represents the Glycoprotein B encoding segment between the primers used to amplify it.

FIG. 24 is an alignment of protein sequences showing an RGD triplet near the N-terminal of mature KSHV Glycoprotein B. The upper panel shows alignment of the Glycoprotein B with RGD domains in other proteins. The lower panel shows predicted signal peptidase cleavage sites for producing the mature form of Glycoprotein B.

DETAILED DESCRIPTION

We have discovered and characterized polynucleotides encoding Glycoprotein B from herpes viruses of the RFHV/KSHV subfamily. The polynucleotides, oligonucleotides, polypeptides and antibodies embodied in this invention are useful in the diagnosis, clinical monitoring, and treatment of herpes virus infections and related conditions.

The source for the polynucleotide for the RFHV Glycoprotein B was affected tissue samples taken from *Macaque nemestrina* monkeys with retroperitoneal fibromatosis ("RF"). The polynucleotide for the KSHV Glycoprotein B was obtained from affected tissue samples taken from humans with Kaposi's Sarcoma ("KS"). The tissues used for the present invention were known to contain genetic material from RFHV or KSHV, because they had previously been used successfully to clone corresponding DNA Polymerase encoding fragments. The amplification of the DNA Polymerase regions have been described in commonly owned U.S. patent application Ser. No. 60/001,148.

In order to amplify the Glycoprotein B sequences from these samples, we designed oligonucleotides from those of other herpes viruses. Glycoprotein B is expected to be less well conserved between herpes viruses, because it is externally exposed on the viral envelope and therefore under selective pressure from the immune system of the hosts they infect. Accordingly, the oligonucleotides were designed from sequences of herpes viruses believed to be most closely related to RFHV and KSHV. These two viruses are known from the DNA polymerase sequences to be closely related gamma type herpes viruses.

Oligonucleotides were designed primarily from Glycoprotein B sequences previously known for four gamma

herpes viruses: sHV1, eHV2, bHV4, mHV68 and hEBV. Comparison of the amino acid sequences of these four Glycoprotein B molecules revealed nine relatively conserved regions. Based on the sequence data, oligonucleotides were constructed comprising a degenerate segment and a consensus segment, as described in a following section. Three of these oligonucleotides have been used as primers in amplification reactions that have yielded fragments of the RFHV and KSHV Glycoprotein B encoding segments from the RF and KS tissue.

The RFHV and KSHV polynucleotide sequence fragments obtained after the final amplification step are shown in FIG. 1 (SEQ ID NO:1 and SEQ. ID NO:3, respectively). Included are segments at each end corresponding to the hybridizing regions of the NIVPA and TVNCB primers used in the amplification. The fragment between the primer binding segments is 319 base pairs in length (residues 36–354), and believed to be an accurate reflection of the sequences of the respective Glycoprotein B encoding regions of the RFHV and KSHV genomes.

The 319 base pair Glycoprotein B encoding polynucleotide segment from RFHV is only 60% identical with that from sHV1 and bHV4, the most closely related sequences from outside the RFHV/KSHV subfamily. The 319 base pair polynucleotide segment from KSHV is only 63% identical with sHV1 and bHV4. The segments are 76% identical between RFHV and KSHV.

Also shown are the corresponding predicted amino acid sequences (SEQ ID NO:2 and SEQ ID NO:4). The polypeptide sequences are novel, and are partly homologous to Glycoprotein B sequences from other herpes viruses. The fragments shown are predicted to be about 1/8 of the entire Glycoprotein B sequence. They begin about 80 amino acids downstream from the predicted N-terminal methionine of the pre-processed protein. There is a potential N-linked glycosylation site at position 80 of the amino acid sequence, according to the sequence Asn-Xaa-(Thr/Ser). This site is conserved between RFHV and KSHV, and is also conserved amongst other known gamma herpes viruses. There is also a cysteine residue at position 58 that is conserved across herpes viruses of the gamma, beta, and alpha subfamilies, which may play a role in maintaining the three-dimensional structure of the protein.

The 106 amino acid segment of Glycoprotein B encoded by the 319 base pairs between the amplification primers is 91% identical between RFHV and KSHV, but only 65% identical between KSHV and that of bHV4, the closest sequence outside the RFHV/KSHV subfamily.

Glycoprotein B molecules expressed by the RFHV/KSHV herpes virus subfamily are expected to have many of the properties described for Glycoprotein B of other herpes viruses. Glycoprotein B molecules are generally about 110 kDa in size, corresponding to about 800–900 amino acids or about 2400–2700 base pairs. Hydrophobicity plots indicate regions from the N terminus to the C terminus in the following order: a hydrophobic region corresponding to a membrane-directing leader sequence; a mixed polarity region corresponding to an extracellular domain; a hydrophobic region corresponding to a transmembrane domain; and another mixed polarity region corresponding to a cytoplasmic domain.

The full sequence of the KSHV Glycoprotein B, shown in FIG. 19, confirms these predictions: The gene encodes about 845 amino acids including the signal peptide and a transmembrane region near the C-terminus. Cysteine residues are conserved with other Glycoprotein B sequences, and an

additional potential disulfide may help stabilize the three-dimensional structure.

Glycoprotein B is generally expressed on the envelope of infectious and defective viral particles, and on the surface of infected cells. It is generally glycosylated, and may comprise 5–20 glycosylation sites or more. It is also generally expressed as a protein dimer, which assembles during translocation to the surface of the host cell, prior to budding of the virus. The site responsible for dimerization appears to be located between about amino acid 475 and the membrane spanning segment (Navarro et al.).

Previous studies have mapped several biochemical functions related to infectivity to different regions of the Glycoprotein B molecule. Glycoprotein B and Glycoprotein C are both implicated in initial binding of HSV1 and bovine herpes virus 1 to target cells (Herold et al., Byrne et al.). The moiety on the cells recognized by Glycoprotein B appears to be heparan sulfate; the binding is inhibitable by fluid-phase heparin. Mutants that lack Glycoprotein C can still bind target cells, but mutants that lack both Glycoprotein C and Glycoprotein B are severely impaired in their ability to gain access to the cells.

Another apparently important function is the ability of Glycoprotein B to promote membrane fusion and entry of the virus into the cell. In human CMV, the fusogenic role appears to map to the first hydrophobic domain of Glycoprotein B, and may be associated with conserved glycine residues within this region (Reschke et al.). In HSV1 mutants, the ability of Glycoprotein B to promote syncytia formation maps to multiple sites in the cytoplasmic domain of the protein, near the C-terminus (Kostal et al.).

In order to exercise some of these more complicated functions, it seems likely that Glycoprotein B associates not only with a second Glycoprotein B molecule, but with other components encoded by the virus. For example, the UMA5 gene product appears to be required for Glycoprotein B induced fusion (Haanes et al.). It has been hypothesized that Glycoprotein B cooperates with other surface proteins to form a hydrophobic fusion pore in the surface of the target cell (Pereira et al.). Glycoprotein B has been found to elicit a potent antibody response capable of neutralizing the intact virus. Monoclonal antibodies with neutralizing activity may be directed against many different sites on the Glycoprotein B molecule.

Consequently, it is expected that the Glycoprotein B molecule bears sites that interact with the target cell, help promote fusion, and associate with other viral proteins. It is predicted that Glycoprotein B molecules of RFHV/KSHV subfamily viruses will perform many of the functions of Glycoprotein B in other species of herpes virus, and bear active regions with some of the same properties. Interfering with any of these active regions with a drug, an antibody, or by mutation, may impair viral infectivity or virulence.

Subsequent to discovery of the Glycoprotein B of RFHV and KSHV, a third member of the RFHV/KSHV subfamily was identified in a sample of affected tissue from a Macaca mulatta (Example 12). This Glycoprotein B is closely related but not identical to RFHV, and is designated RFHV2. It is predicted that other members of the RFHV/KSHV subfamily will emerge, including some that are pathogenic to humans. This disclosure teaches how new members of the subfamily can be detected and characterized.

The homology between Glycoprotein B sequences within the RFHV/KSHV subfamily means that the polynucleotides and polypeptides embodied in this invention are reliable markers amongst different strains of the subfamily. The

polynucleotides, polypeptides, and antibodies embodied in this invention are useful in such applications as the detection and treatment of viral infection in an individual, due to RFHV, KSHV, or other herpes viruses in the same subfamily. The polynucleotides, oligonucleotide probes, polypeptides, antibodies, and vaccine compositions relating to Glycoprotein B, and the preparation and use of these compounds, is described in further detail in the sections that follow.

Abbreviations

The following abbreviations are used herein to refer to species of herpes viruses, and polynucleotides and polypeptides derived therefrom:

TABLE 1

Abbreviations for Herpes Virus Strains		
Designation	Virus	Provisional Subfamily Assignment
RFHV	simian Retroperitoneal Fibromatosis-associated HerpesVirus	gamma-HerpesVirus
KSHV	human Kaposi's Sarcoma-associated HerpesVirus	
mHV68	murine HerpesVirus 68	beta-HerpesVirus
bHV4	bovine Herpesvirus 4	
eHV2	equine HerpesVirus 2	
sHV1	saimiri monkey HerpesVirus 1	
hEBV	human Epstein-Barr Virus	
hCMV	human CytoMegalovirus	
mCMV	murine CytoMegalovirus	
gpCMV	guinea pig CytoMegalovirus	alpha-HerpesVirus
hHV6	human HerpesVirus 6	
hVZV	human Varicella-Zoster Virus	
HSV1	human Herpes Simplex Virus 1	
HSV2	human Herpes Simplex Virus 2	
sHVS A8	simian HerpesVirus A8	
eHV1	equine HerpesVirus 1	gamma-HerpesVirus
iHV1	ictalurid catfish HerpesVirus	

General Definitions

"Glycoprotein B" is a particular protein component of a herpes virus, encoded in the viral genome and believed to be expressed at the surface of the intact virus. Functional studies with certain species of herpes virus, especially HSV1, hCMV, and bovine herpes virus 1, have implicated Glycoprotein B in a number of biochemical functions related to viral infectivity. These include binding to components on the surface of target cells, such as heparan sulfate, fusion of the viral membrane with the membrane of the target cell, penetration of the viral capsid into the cell, and formation of polynucleated syncytial cells. Glycoprotein B has been observed as a homodimer, and may interact with other viral surface proteins in order to exert some of its biochemical functions. Different biochemical functions, particularly heparan sulfate binding and membrane fusion, appear to map to different parts of the Glycoprotein B molecule. A Glycoprotein B molecule of other herpes viruses, including members of the RFHV/KSHV subfamily, may perform any or all of these functions. As used herein, the term Glycoprotein B includes unglycosylated, partly glycosylated, and fully glycosylated forms, and both monomers and polymers.

As used herein, a Glycoprotein B fragment, region, or segment is a fragment of the Glycoprotein B molecule, or a transcript of a subregion of a Glycoprotein B encoding polynucleotide. The intact Glycoprotein B molecule, or the full-length transcript, will exert biochemical functions related to viral activity, such as those described above. Some or all of these functions may be preserved on the fragment, or the fragment may be from a part of the intact molecule which is unable to perform these functions on its own.

"Glycoprotein B activity" refers to any biochemical function of Glycoprotein B, or any biological activity of a herpes virus attributable to Glycoprotein B. These may include but are not limited to binding of the protein to cells, cell receptors such as heparan sulfate, and receptor analogs; viral binding or penetration into a cell, or cell fusion.

The term "Glycoprotein B gene" refers to a gene comprising a sequence that encodes a Glycoprotein B molecule as defined above. It is understood that a Glycoprotein B gene may give rise to processed and altered translation products, including but not limited to forms of Glycoprotein B with or without a signal or leader sequence, truncated or internally deleted forms, multimeric forms, and forms with different degrees of glycosylation.

As used herein, a "DNA Polymerase" is a protein or a protein analog, that under appropriate conditions is capable of catalyzing the assembly of a DNA polynucleotide with a sequence that is complementary to a polynucleotide used as a template. ADNA Polymerase may also have other catalytic activities, such as 3'-5' exonuclease activity; any of the activities may predominate. A DNA Polymerase may require association with additional proteins or co-factors in order to exercise its catalytic function.

"RFHV" is a virus of the herpes family detected in the tissue samples of *Macaque nemestrina* monkeys affected with Retroperitoneal Fibromatosis (RF). RFHV is synonymous with the terms "RFHV1", "RFHVMn", and "RFMn". "KSHV" is a virus of the herpes virus family detected in the tissue samples of humans affected with Kaposi's Sarcoma (KS). A third member of the RFHV/KSHV subfamily is a virus identified in a *%M. mulatta* monkey. The virus is referred to herein as "RFHV2". "RFHV2" is synonymous with the terms "RFHVMm" and "RFMm".

The "RFHV/KSHV subfamily" is a term used herein to refer to a collection of herpes viruses capable of infecting vertebrate species. The subfamily consists of members that have Glycoprotein B sequences that are more closely related to that of the corresponding sequences of RFHV or KSHV than other herpes viruses, including SHV1, eHV2, bHV4, mHV68 and hEBV. Preferably, the polynucleotide encoding Glycoprotein B comprises a segment that is at least 65% identical to that of RFHV (SEQ. ID NO: 1) or KSHV (SEQ. ID NO:3) between residues 36 and 354; or at least about 74% identical to the oligonucleotide SHMDA, or at least about 73% identical to the oligonucleotide CFSSB, or at least about 72% identical to the nucleotide ENTFA, or at least about 80% identical to the nucleotide DNIQB. RFHV and KSHV are exemplary members of the RFHV/KSHV subfamily. The RFHV/KSHV subfamily represents a subset of the gamma subfamily of herpes viruses.

The terms "polynucleotide" and "oligonucleotide" are used interchangeably, and refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Polynucleotides may have any three-dimensional structure, and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: a gene or gene fragment, exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A

polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component.

The term polynucleotide, as used herein, refers to both double- and single-stranded molecules. Unless otherwise specified or required, any embodiment of the invention described herein that is a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form.

In the context of polynucleotides, a "linear sequence" or a "sequence" is an order of nucleotides in a polynucleotide in a 5' to 3' direction in which residues that neighbor each other in the sequence are contiguous in the primary structure of the polynucleotide. A "partial sequence" is a linear sequence of part of a polynucleotide which is known to comprise additional residues in one or both directions.

"Hybridization" refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson-Crick base pairing, Hoogsteen binding, or in any other sequence-specific manner. The complex may comprise two strands forming a duplex structure, three or more strands forming a multi-stranded complex, a single self-hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of a PCR, or the enzymatic cleavage of a polynucleotide by a ribozyme.

Hybridization reactions can be performed under conditions of different "stringency". Conditions that increase the stringency of a hybridization reaction are widely known and published in the art: see, for example, Sambrook Fritsch & Maniatis. Examples of relevant conditions include (in order of increasing stringency): incubation temperatures of 25° C., 37° C., 50° C., and 68° C.; buffer concentrations of 10×SSC, 6×SSC, 1×SSC, 0.1×SSC (where SSC is 0.15 M NaCl and 15 mM citrate buffer) and their equivalent using other buffer systems; formamide concentrations of 0%, 25%, 50%, and 75%; incubation times from 5 min to 24 h; 1, 2, or more washing steps; wash incubation times of 1, 5, or 15 min; and wash solutions of 6×SSC, 1×SSC, 0.1×SSC, or deionized water.

" T_m " is the temperature in degrees Centigrade at which 50% of a polynucleotide duplex made of complementary strands hydrogen bonded in an antiparallel direction by Watson-Crick base pairing dissociates into single strands under the conditions of the experiment. T_m may be predicted according to standard formula; for example:

$$T_m = 81.5 + 16.6 \log [\text{Na}^+] + 0.41(\% \text{ G/C}) - 0.61(\% \text{ F}) - 600/L$$

where $[\text{Na}^+]$ is the cation concentration (usually sodium ion) in mol/L; (% G/C) is the number of G and C residues as a percentage of total residues in the duplex; (% F) is the percent formamide in solution (wt/vol); and L is the number of nucleotides in each strand of the duplex.

A "stable duplex" of polynucleotides, or a "stable complex" formed between any two or more components in a biochemical reaction, refers to a duplex or complex that is sufficiently long-lasting to persist between the formation of the duplex or complex, and its subsequent detection. The duplex or complex must be able to withstand whatever conditions exist or are introduced between the moment of formation and the moment of detection, these conditions being a function of the assay or reaction which is being performed. Intervening conditions which may optionally be

present and which may dislodge a duplex or complex include washing, heating, adding additional solutes or solvents to the reaction mixture (such as denaturants), and competing with additional reacting species. Stable duplexes or complexes may be irreversible or reversible, but must meet the other requirements of this definition. Thus, a transient complex may form in a reaction mixture, but it does not constitute a stable complex if it dissociates spontaneously or as a result of a newly imposed condition or manipulation introduced before detection.

When stable duplexes form in an antiparallel configuration between two single-stranded polynucleotides, particularly under conditions of high stringency, the strands are essentially "complementary". A double-stranded polynucleotide can be "complementary" to another polynucleotide, if a stable duplex can form between one of the strands of the first polynucleotide and the second. A complementary sequence predicted from the sequence of a single stranded polynucleotide is the optimum sequence of standard nucleotides expected to form hydrogen bonding with the single-stranded polynucleotide according to generally accepted base-pairing rules.

A "sense" strand and an "antisense" strand when used in the same context refer to single-stranded polynucleotides which are complementary to each other. They may be opposing strands of a double-stranded polynucleotide, or one strand may be predicted from the other according to generally accepted base-pairing rules. If not specified, the assignment of one or the other strand as "sense" or "antisense" may be arbitrary. In relation to a polypeptide-encoding segment of a polynucleotide, the "sense" strand is generally the strand comprising the encoding segment.

When comparison is made between polynucleotides for degree of identity, it is implicitly understood that complementary strands are easily generated, and the sense or antisense strand is selected or predicted that maximizes the degree of identity between the polynucleotides being compared. For example, where one or both of the polynucleotides being compared is double-stranded, the sequences are identical if one strand of the first polynucleotide is identical with one strand of the second polynucleotide. Similarly, when a polynucleotide probe is described as identical to its target, it is understood that it is the complementary strand of the target that participates in the hybridization reaction between the probe and the target.

A linear sequence of nucleotides is "essentially identical" to another linear sequence, if both sequences are capable of hybridizing to form duplexes with the same complementary polynucleotide. Sequences that hybridize under conditions of greater stringency are more preferred. It is understood that hybridization reactions can accommodate insertions, deletions, and substitutions in the nucleotide sequence. Thus, linear sequences of nucleotides can be essentially identical even if some of the nucleotide residues do not precisely correspond or align. Sequences that correspond or align more closely to the invention disclosed herein are comparably more preferred. Generally, a polynucleotide region of about 25 residues is essentially identical to another region, if the sequences are at least about 85% identical; more preferably, they are at least about 90% identical; more preferably, they are at least about 95% identical; still more preferably, the sequences are 100% identical. A polynucleotide region of 40 residues or more will be essentially identical to another region, after alignment of homologous portions if the sequences are at least about 75% identical; more preferably, they are at least about 80% identical; more preferably, they are at least about 85% identical; even more

preferably, they are at least about 90% identical; still more preferably, the sequences are 100% identical.

In determining whether polynucleotide sequences are essentially identical, a sequence that preserves the functionality of the polynucleotide with which it is being compared is particularly preferred. Functionality can be determined by different parameters. For example, if the polynucleotide is to be used in reactions that involve hybridizing with another polynucleotide, then preferred sequences are those which hybridize to the same target under similar conditions. In general, the T_m of a DNA duplex decreases by about 1° C. for every 1% decrease in sequence identity for duplexes of 200 or more residues; or by about 5° C. for duplexes of less than 40 residues, depending on the position of the mismatched residues (see, e.g., Meinkoth et al.). Essentially identical sequences of about 100 residues will generally form a stable duplex with each other's respective complementary sequence at about 20° C. less than T_m ; preferably, they will form a stable duplex at about 15° C. less; more preferably, they will form a stable duplex at about 10° C. less; even more preferably, they will form a stable duplex at about 5° C. less; still more preferably, they will form a stable duplex at about T_m . In another example, if the polypeptide encoded by the polynucleotide is an important part of its functionality, then preferred sequences are those which encode identical or essentially identical polypeptides. Thus, nucleotide differences which cause a conservative amino acid substitution are preferred over those which cause a non-conservative substitution, nucleotide differences which do not alter the amino acid sequence are more preferred, while identical nucleotides are even more preferred. Insertions or deletions in the polynucleotide that result in insertions or deletions in the polypeptide are preferred over those that result in the down-stream coding region being rendered out of phase; polynucleotide sequences comprising no insertions or deletions are even more preferred. The relative importance of hybridization properties and the encoded polypeptide sequence of a polynucleotide depends on the application of the invention.

A polynucleotide has the same "characteristics" of another polynucleotide if both are capable of forming a stable duplex with a particular third polynucleotide under similar conditions of maximal stringency. Preferably, in addition to similar hybridization properties, the polynucleotides also encode essentially identical polypeptides.

"Conserved" residues of a polynucleotide sequence are those residues which occur unaltered in the same position of two or more related sequences being compared. Residues that are relatively conserved are those that are conserved amongst more related sequences or with a greater degree of identity than residues appearing elsewhere in the sequences.

"Related" polynucleotides are polynucleotides that share a significant proportion of identical residues.

As used herein, a "degenerate" oligonucleotide sequence is a designed sequence derived from at least two related originating polynucleotide sequences as follows: the residues that are conserved in the originating sequences are preserved in the degenerate sequence, while residues that are not conserved in the originating sequences may be provided as several alternatives in the degenerate sequence. For example, the degenerate sequence AYASA may be designed from originating sequences ATACA and ACAGA, where Y is C or T and S is C or G. Y and S are examples of "ambiguous" residues. A degenerate segment is a segment of a polynucleotide containing a degenerate sequence.

It is understood that a synthetic oligonucleotide comprising a degenerate sequence is actually a mixture of closely

related oligonucleotides sharing an identical sequence, except at the ambiguous positions. Such an oligonucleotide is usually synthesized as a mixture of all possible combinations of nucleotides at the ambiguous positions. Each of the oligonucleotides in the mixture is referred to as an “alternative form”. The number of forms in the mixture is equal to

$$\prod_{i=1}^n k_i$$

where k_i is the number of alternative nucleotides allowed at each position.

As used herein, a “consensus” oligonucleotide sequence is a designed sequence derived from at least two related originating polynucleotide sequences as follows: the residues that are conserved in all originating sequences are preserved in the consensus sequence; while at positions where residues are not conserved, one alternative is chosen from amongst the originating sequences. In general, the nucleotide chosen is the one which occurs in the greatest frequency in the originating sequences. For example, the consensus sequence AAAAA may be designed from originating sequences CAAAA, AAGAA, and AAAAT. A consensus segment is a segment of a polynucleotide containing a consensus sequence.

A polynucleotide “fragment” or “insert” as used herein generally represents a sub-region of the full-length form, but the entire full-length polynucleotide may also be included.

Polynucleotides “correspond” to each other if they are believed to be derived from each other or from a common ancestor. For example, encoding regions in the genes of different viruses correspond if they share a significant degree of identity, map to the same location of the genome, or encode proteins that perform a similar biochemical function. Messenger RNA corresponds to the gene from which it is transcribed. cDNA corresponds to the RNA from which it has been produced, and to the gene that encodes the RNA. A protein corresponds to a polynucleotide encoding it, and to an antibody that is capable of binding it specifically.

A “probe” when used in the context of polynucleotide manipulation refers to an oligonucleotide which is provided as a reagent to detect a target potentially present in a sample of interest by hybridizing with the target. Usually, a probe will comprise a label or a means by which a label can be attached, either before or subsequent to the hybridization reaction. Suitable labels include, but are not limited to radioisotopes, fluorochromes, chemiluminescent compounds, dyes, and proteins, including enzymes.

A “primer” is an oligonucleotide, generally with a free 3'-OH group, that binds to a target potentially present in a sample of interest by hybridizing with the target, and thereafter promotes polymerization of a polynucleotide complementary to the target.

Processes of producing replicate copies of the same polynucleotide, such as PCR or gene cloning, are collectively referred to herein as “amplification” or “replication”. For example, single or double-stranded DNA may be replicated to form another DNA with the same sequence. RNA may be replicated, for example, by an RNA-directed RNA polymerase, or by reverse-transcribing the DNA and then performing a PCR. In the latter case, the amplified copy of the RNA is a DNA with the identical sequence.

A “polymerase chain reaction” (“PCR”) is a reaction in which replicate copies are made of a target polynucleotide using one or more primers, and a catalyst of polymerization,

such as a reverse transcriptase or a DNA polymerase, and particularly a thermally stable polymerase enzyme. Generally, a PCR involves reiteratively performing three steps: “annealing”, in which the temperature is adjusted such that oligonucleotide primers are permitted to form a duplex with the polynucleotide to be amplified; “elongating”, in which the temperature is adjusted such that oligonucleotides that have formed a duplex are elongated with a DNA polymerase, using the polynucleotide to which they've formed the duplex as a template; and “melting”, in which the temperature is adjusted such that the polynucleotide and elongated oligonucleotides dissociate. The cycle is then repeated until the desired amount of amplified polynucleotide is obtained. Methods for PCR are taught in U.S. Pat. No. 4,683,195 (Mullis) and U.S. Pat. No. 4,683,202 (Mullis et al.).

A “control element” or “control sequence” is a nucleotide sequence involved in an interaction of molecules that contributes to the functional regulation of a polynucleotide, including replication, duplication, transcription, splicing, translation, or degradation of the polynucleotide. The regulation may affect the frequency, speed, or specificity of the process, and may be enhancing or inhibitory in nature. Control elements are known in the art. For example, a “promoter” is an example of a control element. A promoter is a DNA region capable under certain conditions of binding RNA polymerase and initiating transcription of a coding region located downstream (in the 3' direction) from the promoter.

“Operatively linked” refers to a juxtaposition of genetic elements, wherein the elements are in a relationship permitting them to operate in the expected manner. For instance, a promoter is operatively linked to a coding region if the promoter helps initiate transcription of the coding sequence. There may be intervening residues between the promoter and coding region so long as this functional relationship is maintained.

The terms “polypeptide”, “peptide” and “protein” are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation, such as conjugation with a labeling component.

In the context of polypeptides, a “linear sequence” or a “sequence” is an order of amino acids in a polypeptide in an N-terminal to C-terminal direction in which residues that neighbor each other in the sequence are contiguous in the primary structure of the polypeptide. A “partial sequence” is a linear sequence of part of a polypeptide which is known to comprise additional residues in one or both directions.

A linear sequence of amino acids is “essentially identical” to another sequence if the two sequences have a substantial degree of sequence identity. It is understood that the folding and the biochemical function of proteins can accommodate insertions, deletions, and substitutions in the amino acid sequence. Thus, linear sequences of amino acids can be essentially identical even if some of the residues do not precisely correspond or align. Sequences that correspond or align more closely to the invention disclosed herein are more preferred. It is also understood that some amino acid substitutions are more easily tolerated. For example, substitution of an amino acid with hydrophobic side chains, aromatic side chains, polar side chains, side chains with a

positive or negative charge, or side chains comprising two or fewer carbon atoms, by another amino acid with a side chain of like properties can occur without disturbing the essential identity of the two sequences. Methods for determining homologous regions and scoring the degree of homology are well known in the art; see for example Altschul et al. and Henikoff et al. Well-tolerated sequence differences are referred to as "conservative substitutions". Thus, sequences with conservative substitutions are preferred over those with other substitutions in the same positions; sequences with identical residues at the same positions are still more preferred.

Generally, a polypeptide region will be essentially identical to another region, after alignment of homologous portions, if the sequences are at least about 92% identical; more preferably, they are at least about 95% identical; more preferably, they are at least about 95% identical and comprise at least another 2% which are either identical or are conservative substitutions; more preferably, they are at least about 97% identical; more preferably, they are at least about 97% identical, and comprise at least another 2% which are either identical or are conservative substitutions; more preferably, they are at least about 99% identical; still more preferably, the sequences are 100% identical.

In determining whether polypeptide sequences are essentially identical, a sequence that preserves the functionality of the polypeptide with which it is being compared is particularly preferred. Functionality may be established by different parameters, such as enzymatic activity, the binding rate or affinity in a substrate-enzyme or receptor-ligand interaction, the binding affinity with an antibody, and X-ray crystallographic structure.

A polypeptide has the same "characteristics" of another polypeptide if it displays the same biochemical function, such as enzyme activity, ligand binding, or antibody reactivity. Preferred characteristics of a polypeptide related to a Glycoprotein B or a Glycoprotein B fragment are the ability to bind analogs of the cell surface receptor bound by Glycoprotein B of other herpes species, the ability to promote membrane fusion with a target cell, the ability to promote viral penetration of the host cell. Also preferred is a polypeptide that displays the same biochemical function as the polypeptide with which it is being compared, and in addition, is believed to have a similar three-dimensional conformation, as predicted by computer modeling or determined by such techniques as X-ray crystallography.

The "biochemical function", "biological function" or "biological activity" of a polypeptide includes any feature of the polypeptide detectable by suitable experimental investigation. "Altered" biochemical function can refer to a change in the primary, secondary, tertiary, or quaternary structure of the polypeptide; detectable, for example, by molecular weight determination, circular dichroism, antibody binding, difference spectroscopy, or nuclear magnetic resonance. It can also refer to a change in reactivity, such as the ability to catalyze a certain reaction, or the ability to bind a cofactor, substrate, inhibitor, drug, hapten, or other polypeptide. A substance may be said to "interfere" with the biochemical function of a polypeptide if it alters the biochemical function of the polypeptide in any of these ways.

A "fusion polypeptide" is a polypeptide comprising regions in a different position in the sequence than occurs in nature. The regions may normally exist in separate proteins and are brought together in the fusion polypeptide; or they may normally exist in the same protein but are placed in a new arrangement in the fusion polypeptide. A fusion polypeptide may be created, for example, by chemical

synthesis, or by creating and translating a polynucleotide in which the peptide regions are encoded in the desired relationship.

An "antibody" (interchangeably used in plural form) is an immunoglobulin molecule capable of specific binding to a target, such as a polypeptide, through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term encompasses not only intact antibodies, but also fragments thereof, mutants thereof, fusion proteins, humanized antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity.

"Immunological recognition" or "immunological reactivity" refers to the specific binding of a target through at least one antigen recognition site in an immunoglobulin or a related molecule, such as a B cell receptor or a T cell receptor.

The term "antigen" refers to the target molecule that is specifically bound by an antibody through its antigen recognition site. The antigen may, but need not be chemically related to the immunogen that stimulated production of the antibody. The antigen may be polyvalent, or it may be a monovalent hapten. Examples of kinds of antigens that can be recognized by antibodies include polypeptides, polynucleotides, other antibody molecules, oligosaccharides, complex lipids, drugs, and chemicals.

An "immunogen" is a compound capable of stimulating production of an antibody when injected into a suitable host, usually a mammal. Compounds with this property are described as "immunogenic". Compounds may be rendered immunogenic by many techniques known in the art, including crosslinking or conjugating with a carrier to increase valency, mixing with a mitogen to increase the immune response, and combining with an adjuvant to enhance presentation.

A "vaccine" is a pharmaceutical preparation for human or animal use, which is administered with the intention of conferring the recipient with a degree of specific immunological reactivity against a particular target, or group of targets. The immunological reactivity may be antibodies or cells (particularly B cells, plasma cells, T helper cells, and cytotoxic T lymphocytes, and their precursors) that are immunologically reactive against the target, or any combination thereof. Possible targets include foreign or pathological compounds, such as an exogenous protein, a pathogenic virus, or an antigen expressed by a cancer cell. The immunological reactivity may be desired for experimental purposes, for the treatment of a particular condition, for the elimination of a particular substance, or for prophylaxis against a particular condition or substance. Unless specifically indicated, a vaccine referred to herein may be either a passive vaccine or an active vaccine, or it may have the properties of both.

A "passive vaccine" is a vaccine that does not require participation of the recipient's immune response to exert its effect. Usually, it is comprised of antibody molecules reactive against the target. The antibodies may be obtained from a donor subject and sufficiently purified for administration to the recipient, or they may be produced in vitro, for example, from a culture of hybridoma cells, or by genetically engineering a polynucleotide encoding an antibody molecule.

An "active vaccine" is a vaccine administered with the intention of eliciting a specific immune response within the recipient, that in turn has the desired immunological reactivity against the target. An active vaccine comprises a suitable immunogen. The immune response that is desired

may be either humoral or cellular, systemic or secretory, or any combination of these.

A “reagent” polynucleotide, polypeptide, or antibody, is a substance provided for a reaction, the substance having some known and desirable parameters for the reaction.

A reaction mixture may also contain a “target”, such as a polynucleotide, antibody, or polypeptide that the reagent is capable of reacting with. For example, in some types of diagnostic tests, the amount of the target in a sample is determined by adding a reagent, allowing the reagent and target to react, and measuring the amount of reaction product. In the context of clinical management, a target may also be a cell, collection of cells, tissue, or organ that is the object of an administered substance, such as a pharmaceutical compound. A cell that is a target for a viral infection is one to which a virus preferentially localizes for such purposes as replication or transformation into a latent form.

An “isolated” polynucleotide, polypeptide, protein, antibody, or other substance refers to a preparation of the substance devoid of at least some of the other components that may also be present where the substance or a similar substance naturally occurs or is initially obtained from. Thus, for example, an isolated substance may be prepared by using a purification technique to enrich it from a source mixture. Enrichment can be measured on an absolute basis, such as weight per volume of solution, or it can be measured in relation to a second, potentially interfering substance present in the source mixture. Increasing enrichments of the embodiments of this invention are increasingly more preferred. Thus, for example, a 2-fold enrichment is preferred, 10-fold enrichment is more preferred, 100-fold enrichment is more preferred, 1000-fold enrichment is even more preferred. A substance can also be provided in an isolated state by a process of artificial assembly, such as by chemical synthesis or recombinant expression.

A polynucleotide used in a reaction, such as a probe used in a hybridization reaction, a primer used in a PCR, or a polynucleotide present in a pharmaceutical preparation, is referred to as “specific” or “selective” if it hybridizes or reacts with the intended target more frequently, more rapidly, or with greater duration than it does with alternative substances. Similarly, a polypeptide is referred to as “specific” or “selective” if it binds an intended target, such as a ligand, hapten, substrate, antibody, or other polypeptide more frequently, more rapidly, or with greater duration than it does to alternative substances. An antibody is referred to as “specific” or “selective” if it binds via at least one antigen recognition site to the intended target more frequently, more rapidly, or with greater duration than it does to alternative substances. A polynucleotide, polypeptide, or antibody is said to “selectively inhibit” or “selectively interfere with” a reaction if it inhibits or interferes with the reaction between particular substrates to a greater degree or for a greater duration than it does with the reaction between alternative substrates.

A “pharmaceutical candidate” or “drug candidate” is a compound believed to have therapeutic potential, that is to be tested for efficacy. The “screening” of a pharmaceutical candidate refers to conducting an assay that is capable of evaluating the efficacy and/or specificity of the candidate. In this context, “efficacy” refers to the ability of the candidate to affect the cell or organism it is administered to in a beneficial way: for example, the limitation of the pathology due to an invasive virus.

The “effector component” of a pharmaceutical preparation is a component which modifies target cells by altering their function in a desirable way when administered to a

subject bearing the cells. Some advanced pharmaceutical preparations also have a “targeting component”, such as an antibody, which helps deliver the effector component more efficaciously to the target site. Depending on the desired action, the effector component may have any one of a number of modes of action. For example, it may restore or enhance a normal function of a cell, it may eliminate or suppress an abnormal function of a cell, or it may alter a cell’s phenotype. Alternatively, it may kill or render dormant a cell with pathological features, such as a virally infected cell. Examples of effector components are provided in a later section.

A “cell line” or “cell culture” denotes higher eukaryotic cells grown or maintained in vitro. It is understood that the descendants of a cell may not be completely identical (either morphologically, genotypically, or phenotypically) to the parent cell.

A “host cell” is a cell which has been transformed, or is capable of being transformed, by administration of an exogenous polynucleotide. A “host cell” includes progeny of the original transformant.

“Genetic alteration” refers to a process wherein a genetic element is introduced into a cell other than by natural cell division. The element may be heterologous to the cell, or it may be an additional copy or improved version of an element already present in the cell. Genetic alteration may be effected, for example, by transfecting a cell with a recombinant plasmid or other polynucleotide through any process known in the art, such as electroporation, calcium phosphate precipitation, contacting with a polynucleotide-liposome complex, or by transduction or infection with a DNA or RNA virus or viral vector. The alteration is preferably but not necessarily inheritable by progeny of the altered cell.

An “individual” refers to vertebrates, particularly members of a mammalian species, and includes but is not limited to domestic animals, sports animals, and primates, including humans.

The term “primate” as used herein refers to any member of the highest order of mammalian species. This includes (but is not limited to) prosimians, such as lemurs and lorises; tarsoids, such as tarsiers; new-world monkeys, such as squirrel monkeys (*Saimiri sciureus*) and tamarins; old-world monkeys such as macaques (including *Macaca nemestrina*, *Macaca fascicularis*, and *Macaca fuscata*); hylobatids, such as gibbons and siamangs; pongids, such as orangutans, gorillas, and chimpanzees; and hominids, including humans.

The “pathology” caused by a herpes virus infection is anything that compromises the well-being or normal physiology of the host. This may involve (but is not limited to) destructive invasion of the virus into previously uninfected cells, replication of the virus at the expense of the normal metabolism of the cell, generation of toxins or other unnatural molecules by the virus, irregular growth of cells or intercellular structures (including fibrosis), irregular or suppressed biological activity of infected cells, malignant transformation, interference with the normal function of neighboring cells, aggravation or suppression of an inflammatory or immunological response, and increased susceptibility to other pathogenic organisms and conditions.

“Treatment” of an individual or a cell is any type of intervention in an attempt to alter the natural course of the individual or cell. For example, treatment of an individual may be undertaken to decrease or limit the pathology caused by a herpes virus infecting the individual. Treatment includes (but is not limited to) administration of a composition, such as a pharmaceutical composition, and

may be performed either prophylactically, or therapeutically, subsequent to the initiation of a pathologic event or contact with an etiologic agent.

It is understood that a clinical or biological "sample" encompasses a variety of sample types obtained from a subject and useful in an in vitro procedure, such as a diagnostic test. The definition encompasses solid tissue samples obtained as a surgical removal, a pathology specimen, or a biopsy specimen, tissue cultures or cells derived therefrom and the progeny thereof, and sections or smears prepared from any of these sources. Non-limiting examples are samples obtained from infected sites, fibrotic sites, unaffected sites, and tumors. The definition also encompasses blood, spinal fluid, and other liquid samples of biologic origin, and may refer to either the cells or cell fragments suspended therein, or to the liquid medium and its solutes. The definition also includes samples that have been solubilized or enriched for certain components, such as DNA, RNA, protein, or antibody.

Oligonucleotide primers and probes described herein have been named as follows: The first part of the designation is the single amino acid code for a portion of the conserved region of the polypeptide they are based upon, usually 4 residues long. This is followed with the letter A or B, indicating respectively that the oligonucleotide is complementary to the sense or anti-sense strand of the encoding region. Secondary consensus oligonucleotides used for sequencing and labeling reactions have the letters SQ at the end of the designation.

General techniques

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, "Molecular Cloning: A Laboratory Manual", Second Edition (Sambrook, Fritsch & Maniatis, 1989), "Oligonucleotide Synthesis" (M. J. Gait, ed., 1984), "Animal Cell Culture" (R. I. Freshney, ed., 1987); the series "Methods in Enzymology" (Academic Press, Inc.); "Handbook of Experimental Immunology" (D. M. Weir & C. C. Blackwell, eds.), "Gene Transfer Vectors for Mammalian Cells" (J. M. Miller & M. P. Calos, eds., 1987), "Current Protocols in Molecular Biology" (F. M. Ausubel et al., eds., 1987); and "Current Protocols in Immunology" (J. E. Coligan et al., eds., 1991).

All patents, patent applications, articles and publications mentioned herein, both supra and infra, are hereby incorporated herein by reference.

Polynucleotides encoding Glycoprotein B of the herpes virus RFHV/KSHV subfamily

This invention embodies isolated polynucleotide segments derived from Glycoprotein B genes present in herpes viruses that encode a fragment of a Glycoprotein B polypeptide. The polynucleotides are related to the RFHV/KSHV subfamily of herpes viruses. Exemplary polynucleotides encode Glycoprotein B fragments from either RFHV or KSHV. Preferred fragments include those shown in FIG. 1, and subfragments thereof, obtained as described in the Example section below. Especially preferred is the polynucleotide comprising the sequence between residues 36–354 of SEQ. ID NO:1, SEQ. ID NO:3, or SEQ. ID NO:96, or polynucleotides contained in SEQ. ID NO:92.

The polynucleotide segments of RFHV and KSHV between residues 36 and 354 are 76% identical. Shared residues are indicated in FIG. 1 by "*". The longest subregions that are identically shared between RFHV and KSHV within this segment are 15, 17, and 20 nucleotides in length.

The 319 base pair fragments of RFHV and KSHV between the amplification primer binding sites are more identical to each other than either of them are to that of any previously sequenced herpes virus. The next most closely related sequences are sHV1 and bHV4, which are 63% identical to the corresponding sequence of KSHV, and 60% identical to the corresponding sequence of RFHV. The longest number of consecutive bases shared between the Glycoprotein B fragment and any of the previously sequenced viruses is 14. It is believed that any subfragment of the RFHV or KSHV sequence of 16 base pairs or longer will be unique to the RFHV/KSHV subfamily, or to particular herpes virus species and variants within the subfamily.

This invention embodies subfragments contained in the Glycoprotein B gene of the RFHV/KSHV subfamily, preferably contained in the region corresponding to the 319 base pair fragment between residues 36–354 of SEQ. ID NO:1, SEQ. ID NO:3, or SEQ. ID NO:96, or anywhere in SEQ. ID NO:92. Preferably, the sub-fragments are at least about 16 nucleotides in length; more preferably they are at least 18 nucleotides in length; more preferably they are at least 21 nucleotides in length; more preferably they are at least about 25 nucleotides in length; more preferably they are at least about 35 nucleotides in length; still more preferably they are at least about 50 nucleotides in length; yet more preferably they are at least about 75 nucleotides in length, and even more preferably they are 100 nucleotides in length or more. Also embodied in this invention are polynucleotides comprising the entire open reading frame of each respective herpes virus Glycoprotein B.

The RFHV/KSHV subfamily consists of members that have sequences that are more closely identical to the corresponding sequences of RFHV or KSHV, than RFHV or KSHV are to any other virus listed in Table 1. Preferred members of the family may be identified on the basis of the sequence of the Glycoprotein B gene in the region corresponding to that of FIG. 1. Table 2 provides the degree of sequence identities in this region:

TABLE 2

Sequence Identities Between Glycoprotein B of KSHV and other Herpes Viruses

		Identity to polynucleotide fragment:		
Glycoprotein B Sequence	SEQ. ID NO:	Bases 36–354		
		RFHV (SEQ. ID NO:1)	KSHV (SEQ. ID NO:3)	
			Bases 36–354	
RFHV/KSHV subfamily	RFHV	1	(100%)	76%
	KSHV	3	76%	(100%)
Other gamma herpes viruses	sHV1	5	60%	63%
	bHV4	6	60%	63%
	eHV2	7	52%	54%
	mHV68	8	56%	54%
	hEBV	9	<50%	52%
alpha and beta herpes viruses	hCMV	10	<50%	<50%
	hHV6	11	<50%	<50%
	hVZV	12	<50%	<50%
	HSV1	13	<50%	<50%

The percentage of sequence identity is calculated by first aligning the encoded amino acid sequence, determining the corresponding alignment of the encoding polynucleotide, and then counting the number of residues shared between the sequences being compared at each aligned position. No penalty is imposed for the presence of insertions or deletions, but insertions or deletions are permitted only where required to accommodate an obviously increased

number of amino acid residues in one of the sequences being aligned. Offsetting insertions just to improve sequence alignment are not permitted at either the polypeptide or polynucleotide level. Thus, any insertions in the polynucleotide sequence will have a length which is a multiple of 3. The percentage is given in terms of residues in the test sequence that are identical to residues in the comparison or reference sequence.

Preferred Glycoprotein B encoding polynucleotide sequences of this invention are those derived from the RFHV/KSHV herpes virus subfamily. They include those sequences that are at least 65% identical with the RFHV or KSHV sequence between bases 36 and 354; more preferably, the sequences are at least 67% identical; more preferably, the sequences are at least about 70% identical; more preferably, the sequences are at least about 75% identical; more preferably, the sequences are at least about 80% identical; more preferably, the sequences are at least about 85% identical; more preferably, the sequences are at least about 90% identical; even more preferably, the sequences are over 95% identical. Also included are Glycoprotein B encoding regions that are upstream or downstream of a region fulfilling the identity criteria indicated.

Other preferred Glycoprotein B encoding polynucleotide sequences may be identified by the percent identity with RFHV/KSHV subfamily-specific oligonucleotides (Type 2 oligonucleotides) described in more detail in a later section. The percent identity of RFHV and KSHV Glycoprotein B with exemplary Type 2 oligonucleotides is shown in Table 3:

TABLE 3

Sequence Identities Between Glycoprotein B of Select Herpes Viruses and RFHV/KSHV Subfamily Specific Oligonucleotides					
Glycoprotein B Sequence	SEQ ID NO:	Identity to SHMDA (SEQ ID NO:41)	Identity to CFSSB (SEQ ID NO:43)	Identity to ENTFA (SEQ ID NO:45)	Identity to DNIQB (SEQ ID NO:46)
RFHV	1	91%	91%	89%	91%
KSHV	3	100%	85%	89%	97%
sHV1	5	71%	70%	66%	66%
bHV4	6	57%	64%	69%	74%
eHV2	7	57%	61%	54%	60%
mHV68	8	<50%	55%	54%	77%
hEBV	9	57%	55%	60%	51%
hCMV	10	57%	55%	60%	51%
hHV6	11	<50%	52%	60%	57%
hVZV	12	54%	58%	66%	57%
HSV1	13	57%	60%	54%	54%

Percent identity is calculated for oligonucleotides of this length by not allowing gaps in either the oligonucleotide or the polypeptide for purposes of alignment. Throughout this disclosure, whenever at least one of two sequences being compared is a degenerate oligonucleotide comprising an ambiguous residue, the two sequences are identical if at least one of the alternative forms of the degenerate oligonucleotide is identical to the sequence with which it is being compared. As an illustration, AYAAA is 100% identical to ATAAA, since AYAAA is a mixture of ATAAA and ACAAA.

Preferred Glycoprotein B encoding sequences are those which over the corresponding region are at least 72% identical to SHMDA; more preferably they are at least 74% identical; more preferably they are at least about 77% identical; more preferably they are at least about 80% identical; more preferably they are at least about 85% identical; more preferably they are at least about 91% identical. Other preferred Glycoprotein B encoding

sequences are those which over the corresponding region are at least 71% identical to CFSSB; more preferably they are at least 73% identical; more preferably they are at least about 77% identical; more preferably they are at least about 80% identical; more preferably they are at least about 85% identical. Other preferred Glycoprotein B encoding sequences are those which over the corresponding region are at least 70% identical to ENTFA; more preferably they are at least 72% identical; more preferably they are at least about 75% identical; more preferably they are at least about 80% identical; more preferably they are at least about 85% identical; even more preferably, they are at least about 89% identical. Other preferred Glycoprotein B encoding sequences are those which over the corresponding region are at least about 78% identical to DNIQB; more preferably they are at least 80% identical; more preferably they are at least about 85% identical; more preferably they are at least about 91% identical. Also included are Glycoprotein B encoding regions that are upstream or downstream of a region fulfilling the identity criteria indicated.

Glycoprotein B encoding sequences from members of the RFHV/KSHV subfamily identified by any of the aforementioned sequence comparisons, using either RFHV or KSHV sequences, or the subfamily-specific oligonucleotides, are equally preferred. Exemplary sequences are the Glycoprotein B encoding sequences of RFHV and KSHV. Also embodied in this invention are fragments of any Glycoprotein B encoding sequences of the subfamily, and longer polynucleotides comprising such polynucleotide fragments.

The polynucleotide sequences described in this section provide a basis for obtaining the synthetic oligonucleotides, proteins and antibodies outlined in the sections that follow. These compounds may be prepared by standard techniques known to a practitioner of ordinary skill in the art, and may be used for a number of investigative, diagnostic, and therapeutic purposes, as described below.

Preparation of polynucleotides

Polynucleotides and oligonucleotides of this invention may be prepared by any suitable method known in the art. For example, oligonucleotide primers can be used in a PCR amplification of DNA obtained from herpes virus infected tissue, as in Example 3 and Example 11, described below. Alternatively, oligonucleotides can be used to identify suitable bacterial clones of a DNA library, as described below in Example 8.

Polynucleotides may also be prepared directly from the sequence provided herein by chemical synthesis. Several methods of synthesis are known in the art, including the triester method and the phosphite method. In a preferred method, polynucleotides are prepared by solid-phase synthesis using mononucleoside phosphoramidite coupling units. See, for example Horise et al., Beaucage et al., Kumar et al., and U.S. Pat. No. 4,415,732.

A typical solid-phase synthesis involves reiterating four steps: deprotection, coupling, capping, and oxidation. This results in the stepwise synthesis of an oligonucleotide in the 3' to 5' direction.

In the first step, the growing oligonucleotide, which is attached at the 3'-end via a (—O—) group to a solid support, is deprotected at the 5' end. For example, the 5' end may be protected by a —ODMT group, formed by reacting with 4,4'-dimethoxytrityl chloride (DMT-Cl) in pyridine. This group is stable under basic conditions, but is easily removed under acidic conditions, for example, in the presence of dichloroacetic acid (DCA) or trichloroacetic acid (TCA). Deprotection provides a 5' -OH reactive group.

In the second step, the oligonucleotide is reacted with the desired nucleotide monomer, which itself has first been

converted to a 5'-protected, 3'-phosphoramidite. The 5'-OH of the monomer may be protected, for example, in the form of a —ODMT group, and the 3'-OH group may be converted to a phosphoramidite, such as —OP(OR')NR₂; where R is the isopropyl group —CH(CH₃)₂; and R' is, for example, —H (yielding a phosphoramidite diester), or —CH₃, —CH₂CH₃, or the beta-cyanoethyl group —CH₂CH₂CN (yielding a phosphoramidite triester). The 3'-phosphoramidite group of the monomer reacts with the 5'-OH group of the growing oligonucleotide to yield the phosphite linkage 5'-OP(OR')O-3'.

In the third step, oligonucleotides that have not coupled with the monomer are withdrawn from further synthesis to prevent the formation of incomplete polymers. This is achieved by capping the remaining 5'-OH groups, for example, in the form of acetates (—OC(O)CH₃) by reaction with acetic anhydride (CH₃C(O)—O—C(O)CH₃).

In the fourth step, the newly formed phosphite group (i.e., 5'-OP(OR')O-3') is oxidized to a phosphate group (i.e., 5'-OP(=O)(OR')O-3'); for example, by reaction with aqueous iodine and pyridine.

The four-step process may then be reiterated, since the oligonucleotide obtained at the end of the process is 5'-protected and is ready for use in step one. When the desired full-length oligonucleotide has been obtained, it may be cleaved from the solid support, for example, by treatment with alkali and heat. This step may also serve to convert phosphate triesters (i.e., when R' is not —H) to the phosphate diesters (—OP(=O)₂O—), and to deprotect base-labile protected amino groups of the nucleotide bases.

Polynucleotides prepared by any of these methods can be replicated to provide a larger supply by any standard technique, such as PCR amplification or gene cloning. Cloning and expression vectors comprising a Glycoprotein B encoding polynucleotide

Cloning vectors and expression vectors are provided in this invention that comprise a sequence encoding a herpes virus Glycoprotein B or variant or fragment thereof. Suitable cloning vectors may be constructed according to standard techniques, or may be selected from the large number of cloning vectors available in the art. While the cloning vector selected may vary according to the host cell intended to be used, useful cloning vectors will generally have the ability to self-replicate, may possess a single target for a particular restriction endonuclease, and may carry genes for a marker that can be used in selecting transfected clones. Suitable examples include plasmids and bacterial viruses; e.g., pUC18, mp18, mp19, pBR322, pMB9, ColE1, pCR1, RP4, phage DNAs, and shuttle vectors like pSA3 and pAT28.

Expression vectors generally are replicable polynucleotide constructs that encode a polypeptide operatively linked to suitable transcriptional and translational controlling elements. Examples of transcriptional controlling elements are promoters, enhancers, transcription initiation sites, and transcription termination sites. Examples of translational controlling elements are ribosome binding sites, translation initiation sites, and stop codons. Protein processing elements may also be included: for example, regions that encode leader or signal peptides and protease cleavage sites required for translocation of the polypeptide across the membrane or secretion from the cell. The elements employed would be functional in the host cell used for expression. The controlling elements may be derived from the same Glycoprotein B

gene used in the vector, or they may be heterologous (i.e., derived from other genes and/or other organisms).

Polynucleotides may be inserted into host cells by any means known in the art. Suitable host cells include bacterial cells such as *E. coli*, mycobacteria, other prokaryotic microorganisms and eukaryotic cells (including fungal cells, insect cells, plant cells, and animal cells). The cells are transformed by inserting the exogenous polynucleotide by direct uptake, endocytosis, transfection, f-mating, or electroporation. Subsequently, the exogenous polynucleotide may be maintained within the cell as a non-integrated vector, such as a plasmid, or may alternatively be integrated into the host cell genome.

Cloning vectors may be used to obtain replicate copies of the polynucleotides they contain, or as a means of storing the polynucleotides in a depository for future recovery. Expression vectors and host cells may be used to obtain polypeptides transcribed by the polynucleotides they contain. They may also be used in assays where it is desirable to have intact cells capable of synthesizing the polypeptide, such as in a drug screening assay.

Synthetic Type 1 oligonucleotides for Glycoprotein B of gamma herpes virus

Oligonucleotides designed from sequences of herpes virus Glycoprotein B, as embodied in this invention, can be used as probes to identify related sequences, or as primers in an amplification reaction such as a PCR.

Different oligonucleotides with different properties are described in the sections that follow. Oligonucleotides designated as Type 1 are designed from previously known gamma herpes virus Glycoprotein B polynucleotide sequences. They are designed to hybridize with polynucleotides encoding any gamma herpes virus Glycoprotein B, and may be used to detect previously known species of gamma herpes virus. They may also be used to detect and characterize new species of gamma herpes virus. Oligonucleotides designated as Type 2 are designed from the RFHV and KSHV Glycoprotein B polynucleotide sequences together. They are designed to hybridize with polynucleotides encoding Glycoprotein B of the RFHV/KSHV subfamily, including but not limited to RFHV and KSHV. Oligonucleotides designated as Type 3 are designed from RFHV or KSHV Glycoprotein B sequences that are relatively unique to the individual virus. They are designed to hybridize specifically with polynucleotides encoding Glycoprotein B only from RFHV or KSHV and closely related viral strains.

Some preferred examples of Type 1 oligonucleotides are listed in Table 4. These oligonucleotides have a specificity for Glycoprotein B encoding polynucleotides of a broad range of herpes viruses.

TABLE 4

Type 1 Oligonucleotides used for Detecting, Amplifying, or Characterizing Herpes Virus Polynucleotides encoding Glycoprotein B					
Target: Herpes Glycoprotein B, especially from gamma Herpes Viruses					
Designation	Sequence (5' to 3')	Length	No. of forms	Orientation	SEQ ID:
FRFDA	GCTGTTTCAGATTGACTTAGAYMANMCNTGYCC	33	256	sense	13
NIVPA	GTGTACAAGAAGAACATCGTGCCNTAYATNTTYA A	32	64	sense	14
NIVPASQ	GTGTACAAGAAGAACATCGTGCC	23	1		15
TVNCB	AACATGTCTACAATCTCACARITNACNGTNGT	32	128	anti-sense	16
TVNCBSQ	AACATGTCTACAATCTCACA	20	1		17
FAYDA	AATAACCTCTTTACGGCCCAAATTCARTWYGCN TAYGA	38	64	sense	18
IYGKA	CCAACGAGTGTGATGTGAGCCATTAYGGNAAR CCNGT	38	64	sense	19
IYGKASQ	CCAACGAGTGTGATGTGAGCC	21	1		20
CYSRA	TGCTACTCGCGACCTCTAGTCACCTTYAARTTYR TNAA	38	64	sense	21
CYSRASQ	TGCTACTCGCGACCTCTAGTCACC	24	1		22
NIDFB	ACCGGAGTACAGTTCCACTGTYTTTRAARTCDATR TT	36	48	anti-sense	23
NIDFBSQ	TGTCACCTTGACATGAGGCCA	21	1		24
FREYA	TTTGACCTGGAGACTATGTTYMGNGARTAYAA	32	64	sense	25
FREYB	GCTCTGGGTGTAGTAGTTRTAYTCYCTRAACAT	33	16	anti-sense	26
NVFDB	TCTCGGAACATGCTCTCCAGRTCRAAMACRIT	32	32	anti-sense	27
GGMA	ACCTTCATCAAAAAATCCCTTNGGNGGNATGYT	32	128	sense	28
TVNCA	TGGACTTACAGGACTCGAACNACNGTNAAYTG	32	128	sense	29

The orientation indicated in Table 4 is relative to the encoding region of the polynucleotide. Oligomers with a “sense” orientation will hybridize to the strand antisense to the coding strand and initiate amplification in the direction of the coding sequence. Oligomers with an “antisense” orientation will hybridize to the coding strand and initiate amplification in the direction opposite to the coding sequence.

These oligonucleotides have been designed with several properties in mind: 1) sensitivity for target DNA even when present in the source material at very low copy numbers; 2) sufficient specificity to avoid hybridizing with unwanted sequences; for example, host sequences with limited similarity; 3) sufficient cross-reactivity so that differences between an unknown target and the sequence used to design it do not prevent the oligonucleotide from forming a stable duplex with the target.

For some applications, a particularly effective design is oligonucleotides that have a degenerate segment at the 3' end, designed from a region of at least 2 known polynucleotides believed to be somewhat conserved with the polynucleotide target. The various permutations of the ambiguous residues help ensure that at least one of the alternative forms of the oligonucleotide will be able to hybridize with the target. Adjacent to the degenerate segment at the 5' end of the oligonucleotide is a consensus segment which strengthens any duplex which may form and permits hybridization or amplification reactions to be done at higher temperatures. The degenerate segment is located at the 3' end of the molecule to increase the likelihood of a close match between the oligonucleotide and the target at the site where elongation begins during a polymerase chain reaction.

The ambiguous residues in the degenerate part of the sequences are indicated according to the following code:

TABLE 5

Single Letter Codes for Ambiguous Positions	
Code	Represents
R	A or G (purine)
Y	C or T (pyrimidine)
W	A or T
S	C or G
M	A or C
K	G or T
B	C or G or T (not A)
D	A or G or T (not C)
H	A or C or T (not G)
V	A or C or G (not T)
N	A or C or G or T

The Type 1 oligonucleotides shown in Table 4 are generally useful for hybridizing with Glycoprotein B encoding polynucleotide segments. This may be conducted to detect the presence of the polynucleotide, or to prime an amplification reaction so that the polynucleotide can be characterized further. Suitable targets include polynucleotides encoding a region of a Glycoprotein B from a wide spectrum of gamma herpes viruses, including members of the RFHV/KSHV subfamily. Suitable are those infecting any vertebrate animal, including humans and non-human primates, whether or not the Glycoprotein B or the virus has been previously known or described. Non-limiting examples include polynucleotides encoding Glycoprotein B from any of the gamma herpes viruses listed in Table 1.

The oligonucleotides may be used, inter alia, to prime a reaction to amplify a region of the target polynucleotide in the 3' direction from the site where the oligonucleotide hybridizes. FRFDA, HIVPA, TVNCB, FAYDA, IYGKA, CYSRA, NIDFB, FREYA, FREYB, NVFDB, GGMA, and

TVNCA are oligonucleotides with a consensus segment adjoining a degenerate segment, and are useful for this purpose.

FIG. 2 shows the position along the Glycoprotein B polynucleotide sequence of the RFHV/KSHV subfamily where the aforementioned oligonucleotide primers are expected to hybridize. The map is not drawn to scale, but accurately depicts the order of the predicted hybridization sites in the 5' to 3' direction along the Glycoprotein B encoding strand. Also indicated are approximate lengths of amplification products that may be generated by using various sets of primers in an amplification reaction. The lengths shown include the primer binding sites at each end, and the polynucleotide encompassed between them.

A preferred source of DNA for use as a target for the oligonucleotides of Table 4 is any biological sample (including solid tissue and tissue cultures), particularly of vertebrate animal origin, known or suspected to harbor a herpes virus. DNA is extracted from the source by any method known in the art, including extraction with organic solvents or precipitation at high salt concentration.

A preferred method of amplification is a polymerase chain reaction: see generally U.S. Pat. No. 4,683,195 (Mullis) and U.S. Pat. No. 4,683,202 (Mullis et al.); see U.S. Pat. No. 5,176,995 (Sninsky et al.) for application to viral polynucleotides. An amplification reaction may be conducted by combining the target polynucleotide to be amplified with short oligonucleotides capable of hybridizing with the target and acting as a primer for the polymerization reaction. Also added are substrate mononucleotides and a heat-stable DNA-dependent Glycoprotein B, such as Taq. The conditions used for amplification reactions are generally known in the art, and can be optimized empirically using sources of known viruses, such RFHV, KSHV, hEBV or HSV1. Conditions can be altered, for example, by changing the time and temperature of the amplification cycle, particularly the hybridization phase; changing the molarity of the oligonucleotide primers; changing the buffer composition; and changing the number of amplification cycles. Fine-tuning the amplification conditions is a routine matter for a practitioner of ordinary skill in the art.

In one method, a single primer of this invention is used in the amplification, optionally using a second primer, such as a random primer, to initiate replication downstream from the first primer and in the opposite direction. In a preferred method, at least two of the primers of this invention are used in the same reaction to initiate replication in opposite directions. The use of at least two specific primers enhances the specificity of the amplification reaction, and defines the size of the fragment for comparison between samples. For example, amplification may be performed using primers NIVPA and TVNCB. More preferred is the use of several sets of primers in a nested fashion to enhance the amplification. Nesting is accomplished by performing a first amplification using primers that generate an intermediate product, comprising one or more internal binding sites for additional primers. This is followed by a second amplification, using a new primer in conjunction with one from the previous set, or two new primers. The second amplification product is therefore a subfragment of the first product. If desired, additional rounds of amplification can be performed using additional primers.

Accordingly, nesting amplification reactions can be performed with any combination of three or more oligonucleotide primers comprising at least one primer with a sense orientation and one primer with an antisense orientation. Preferably, primers are chosen so that intermediate ampli-

fication products are no more than about 2000 base pairs; more preferably, they are no more than about 1500 base pairs; even more preferably, they are no more than about 750 base pairs. Preferably, the innermost primers provide a final amplification product of no more than about 1200 base pairs; more preferably, they are no more than about 750 base pairs; even more preferably, they are no more than about 500 base pairs. Accordingly, a preferred combination is at least three primers selected from FAYDA, IYGKA, CYSRA, NIDFB, NVFDB, and FREYB. Another preferred combination is at least three primers selected from FRFDA, NIVPA, TVNCA, NIDFB, NVFDB, and FREYB.

Particularly preferred is a first amplification using primer FRFDA and TVNCB, followed by a second amplification using primer NIVPA and TVNCB. When performed on a polynucleotide from a Glycoprotein B gene of KSHV, the size of the final fragment including the primer binding regions is about 386 bases.

The amplified polynucleotides can be characterized at any stage during the amplification reaction, for example, by size determination. Preferably, this is performed by running the polynucleotide on a gel of about 1–2% agarose. If present in sufficient quantity, the polynucleotide in the gel can be stained with ethidium bromide and detected under ultraviolet light. Alternatively, the polynucleotide can be labeled with a radioisotope such as ^{32}P or ^{35}S before loading on a gel of about 6% polyacrylamide, and the gel can subsequently be used to produce an autoradiogram. A preferred method of labeling the amplified polynucleotide is to end-label an oligonucleotide primer such as NIVPA with ^{32}P using a polynucleotide kinase and gamma- ^{32}P -ATP, and continuing amplification for about 5–15 cycles.

If desired, size separation may also be used as a step in the preparation of the amplified polynucleotide. This is particularly useful when the amplification mixture is found to contain artifact polynucleotides of different size, such as may have arisen through cross-reactivity with undesired targets. A separating gel, such as described in the preceding paragraph, is dried onto a paper backing and used to produce an autoradiogram. Positions of the gel corresponding to the desired bands on the autoradiogram are cut out and extracted by standard techniques. The extracted polynucleotide can then be characterized directly, cloned, or used for a further round of amplification.

The oligonucleotides NIVPASQ, TVNCBSQ, IYGKASQ, CYSRASQ, and NIDFBSQ are each derived from a consensus-degenerate Type 1 oligonucleotide. They retain the consensus segment, but lack the degenerate segment. They are useful, inter alia, in sequencing of a Glycoprotein B polynucleotide fragment successfully amplified using a consensus-degenerate oligonucleotide.

Unwanted polynucleotides in a mixture from an amplification reaction can also be proportionally reduced by shifting to primers of this type. For example, an initial 3–5 cycles of amplification can be conducted using primers NIVPA and TVNCB at $\frac{1}{5}$ to $\frac{1}{25}$ the normal amount. Then a molar excess (for example, 50 pmol) of NIVPASQ and/or TVNCBSQ is added, and the amplification is continued for an additional 30–35 cycles. This reduces the complexity of the oligonucleotides present in the amplification mixture, and permits the reaction temperatures to be increased to reduce amplification of unwanted polynucleotides.

Type 2 oligonucleotide primers for Glycoprotein B of the RFHV/KSHV subfamily

Type 2 oligonucleotides are intended for detection or amplification reactions for the Glycoprotein B of any virus of the RFHV/KSHV subfamily. They are designed from

segments of the Glycoprotein B encoding region that are relatively well conserved between RFHV and KSHV, but not other previously sequenced gamma herpes viruses. Preferred examples are shown in Table 6:

TABLE 6

Type 2 Oligonucleotides used for Detecting, Amplifying, or Characterizing Herpes Virus Polynucleotides encoding Glycoprotein B Target: Glycoprotein B from the RFHV/KSHV subfamily of herpes viruses					
Designation	Sequence (5' to 3')	Length	No. of forms	Orientation	SEQ ID:
SHMDA	AGACCCGTGCCACTCTATGARATHAGYCAAT GGA	35	24	sense	41
SHMDASQ	AGACCCGTGCCACTCTATGA	20	1		42
CFSSB	GTTCACAACAATCTTCATNGARCTRAARCA	30	32	anti-sense	43
CFSSBSQ	GTTCACAACAATCTTCAT	18	1		44
ENTFA	GTCAACGGAGTAGARAAYACNTTYACNGA	29	128	sense	45
DNIQB	ACTGGCTGGCTAAAGTACCTTTGAATRTTRIC NGT	35	16	anti-sense	46
DNIQBSQ	ACTGGCTGGCTAAAGTACCTTTG	23	1		47

Type 2 oligonucleotides may be used for many purposes where specificity for the RFHV/KSHV subfamily specificity is desired. This includes the detection or amplification of Glycoprotein B from known viruses of the RFHV/KSHV subfamily, or characterization of Glycoprotein B from new members of the family.

SHMDA, CFSSB, ENTFA, and DNIQB are consensus-degenerate oligonucleotides with a degenerate 3' end, useful as initial primers for PCR amplifications, including polynucleotides of the RFHV/KSHV subfamily which are not identical to either RFHV or KSHV. SHMDASQ, CFSSBSQ, and DNIQBSQ contain only a consensus segment, and are useful for example in labeling or sequencing polynucleotides already amplified using the consensus-degenerate oligonucleotides.

In one application, these Type 2 oligonucleotides are used individually or in combination as amplification primers. In one example of this application, the oligonucleotides are used directly on DNA obtained from a tissue sample to obtain a Glycoprotein B from the RFHV/KSHV subfamily, but not more distantly related viruses that may be present in the same tissue, such as hEBV, hCMV or HSV1. Thus, SHMDA and DNIQB may be used as primers in a PCR,

optionally preamplified using Type 1 oligonucleotides such as NIVPA and TVNCB. Other combinations are also suitable. In another example, one of the Type 2 oligonucleotides of Table 6 is used in combination with a suitable Type 1

oligonucleotide listed earlier. Thus, NIVPA may be used in combination with DNIQB, or SHMDA may be used in combination with TVNCB as primers in a PCR. The DNA source may optionally be preamplified using NIVPA and TVNCB. Other combinations are also suitable.

In another application, Type 2 oligonucleotides, or oligonucleotides comprising these sequences or fragments thereof, are used as probes in a detection assay. For example, they can be provided with a suitable label such as ³²P, and then used in a hybridization assay with a suitable target, such as DNA amplified using FRFDA and/or NIVPA, along with TVNCB.

Type 3 oligonucleotide primers specific for Glycoprotein B of RFHV or KSHV

Type 3 oligonucleotides are intended for detection or amplification reactions specific for a particular virus. They are non-degenerate segments of the Glycoprotein B encoding region of RFHV or KSHV that are relatively more variable between these two viruses and against other herpes viruses than are other segments of the region. Preferred examples are shown in Table 7, and in the Example section.

TABLE 7

Type 3 Oligonucleotides used for Detecting, Amplifying, or Characterizing Herpes Virus Polynucleotides encoding Glycoprotein B					
Designation	Sequence (5' to 3')	Length	No. of forms	Orientation	SEQ ID:
Target: Glycoprotein B from RFHV					
GMTEB	TGCTGCTTCTGTCATACCGCG	21	1	anti-sense	48
AAITB	TATTTGTTGTGATTGCTGCT	21	1	anti-sense	49
GMTEA	GCGGTATGACAGMGCAGCAA	21	1	sense	50
KYEIA	AACAAATATGAGATCCCCAGG	21	1	sense	51
TDRDB	TCATCCCGATCGGTGAACGTA	21	1	anti-sense	52
VEGLB	TTGTCAGTTAGACCTTCGACG	21	1	anti-sense	53
VEGLA	CCCGTCGAAGGTCTAACTGAC	21	1	sense	54
PVLYA	AGCCAACCAAGTACTGTACTCT	21	1	sense	55
Target: Glycoprotein B from KSHV					
GLTEB	TGATGGCGGACTCTGTCAAGC	21	1	anti-sense	56
TNKYB	GTTCATACTTGTTGGTGATGG	21	1	anti-sense	57
GLTEA	GGGCTTGACAGAGTCCGCCAT	21	1	sense	58

TABLE 7-continued

Type 3 Oligonucleotides used for Detecting, Amplifying, or Characterizing Herpes Virus Polynucleotides encoding Glycoprotein B					
Designation	Sequence (5' to 3')	Length	No. of forms	Orien-tation	SEQ ID:
YELPA	ACAAGTATGAACCTCCGAGAC	21	1	sense	59
VNVNB	ACCCCGTTGACATTACCTTC	21	1	anti-sense	60
TFDVB	TCGTCTCTGTACAGTAAATGTG	21	1	anti-sense	61
TVFLA	CCACAGTATTCTCCAACCAG	21	1	sense	62
SQPVA	GGTACTTTAGCCAGCCGGTCA	21	1	sense	63

GMTEB, AAITB, GMTEA, KYEIA, TDRDB, VEGLB, 15
VEGLA, and PVLVA are specific non-degenerate oligo-
nucleotides for the RFHV Glycoprotein B, and can be used
for the amplification or detection of Glycoprotein B encod-
ing polynucleotides of RFHV origin. Amplification is pref-
erably done using the oligonucleotides in a nested fashion:
e.g., a first amplification is conducted using GMTEA and
VEGLB as primers; then a second amplification is con-
ducted using KYEIA and TDRDB as primers. This provides
an extremely sensitive amplification assay that is specific for
RFHV Glycoprotein B. GMTEB and AAITB hybridize near the
5' end of the fragment, and may be used in combination
with up-stream hybridizing Type 1 oligonucleotides to
amplify or detect sequences in the 5' direction. VEGLA and
PVLVA hybridize near the 3' end of the fragment, and may
be used in combination with down-stream hybridizing Type
1 oligonucleotides to amplify or detect sequences in the 3'
direction.

Similarly, GLTEB, TNKYB, GLTEA, YELPA, VNVNB,
ENTFB, SQPVA, and TVFLA are specific non-degenerate
oligonucleotides for the KSHV Glycoprotein B, and can be
used in a similar fashion, including as primers for an
amplification reaction. Preferably, the amplification is done
using the oligonucleotides in a nested fashion: e.g., a first
amplification is conducted using GLTEA and ENTFB as
primers; then a second amplification is conducted using
YELPA and VNVNB as primers. This provides an extremely
sensitive amplification assay that is specific for KSHV
Glycoprotein B. GLTEB and TNKYB hybridize near the 5'
end of the fragment, and may be used in combination with
up-stream hybridizing Type 1 oligonucleotides to amplify or
detect sequences in the 5' direction. SQPVA and TVFLA
hybridize near the 3' end of the fragment, and may be used
in combination with down-stream hybridizing Type 1 oli-
gonucleotides to amplify or detect sequences in the 3'
direction.

Practitioners skilled in the art will immediately recognize
that oligonucleotides of Types 1, 2 and 3 (in particular, those
shown in Tables 4, 6 and 7) can be used in combination with
each other in a PCR to amplify different sections of a
Glycoprotein B encoding polynucleotide. The specificity of
the amplification reaction generally is determined by the
primer with the least amount of cross reactivity. The size and
location of the amplified fragment is determined by the
primers used in the final round of amplification. For
example, NIVPA used in combination with SQPVB will
amplify about 310 bases of Glycoprotein B encoding poly-
nucleotide from a virus closely related to KSHV. Suitable
combinations of oligonucleotides may be used as ampli-
fication primers in a nested fashion.
Use of synthetic oligonucleotides to characterize polynucle-
otide targets

As described in the previous section, the oligonucleotides
embodied in this invention, can be used as primers for

amplification of polynucleotides encoding a herpes virus
Glycoprotein B, particularly in a polymerase chain reaction.

The conditions for conducting the PCR depend on the
nature of the oligonucleotide being used. In particular, when
using oligonucleotides comprising a degenerate segment, or
a consensus segment that is only partly identical to the
corresponding segment of the target, and when the target
polynucleotide comprises an unknown sequence, the selec-
tion of conditions may be important to the success of the
amplification. Optimizing conditions for a new primer or
new polynucleotide target are routine for a practitioner of
ordinary skill. What follows is a guide to assist in that
objective.

First, the temperature of the annealing step of the PCR is
optimized to increase the amount of target polynucleotide
being amplified above the amount of unrelated polynucle-
otide amplified. Ideally, the temperature permits the primers
to hybridize with the target sequence but not with other
sequences. For primers comprising a consensus segment
(Type 1), the temperature of the annealing step in repeat
cycles of a PCR is generally at least about 45° C.; preferably
it is at least about 50° C. It is also preferable to conduct the
first few cycles of the PCR at even higher temperatures, such
as 55° C. or even 60° C. The higher temperature will compel
the annealing to be more sequence specific during the cycle
and will reduce the background amplification of unrelated
sequences. Annealing steps for subsequent cycles may be
performed under slightly less stringent conditions to
improve the rate of amplification. In an especially preferred
procedure, the first PCR amplification cycle comprises an
annealing step of about 1 min conducted at 60° C. Annealing
steps in subsequent cycles are conducted at 2° C. lower each
cycle, until a temperature of 50° C. is reached. Further
cycles are then conducted with annealing steps at 50° C.,
until the desired degree of amplification is achieved.

Primers which are virus-specific and do not contain a
consensus segment (Type 3) are more selective, and may be
effective over a broader temperature range. Preferred tem-
peratures for the annealing step in PCR amplification cycles
are between 50° C. and 65° C.

Second, the buffer conditions are optimized. We have
found that buffers supplied with commercial preparations of
Taq polymerase are sometimes difficult to use, in part
because of a critical dependence on the concentration of
magnesium ion. PCRs performed using the oligonucleotides
of this invention generally are more easily performed using
a buffer such as that suggested by M. Wigler (Lisitsyn et al.).
Preferably, the final PCR reaction mixture contains (NH₄)
₂SO₄ instead of KCl as the principal ion source. Preferably,
the concentration of (NH₄)₂SO₄ in the final reaction mixture
is about 5–50 mM, more preferably about 10–30 mM, even
more preferably 16 mM. The buffering component is pref-
erably Tris, preferably at a final concentration of about 67
mM and a pH of about 8.8. Under these conditions, the

MgCl₂ concentration is less critical. Preferably the final concentration is about 1–10 mM, more preferably it is about 3–6 mM, optimally it is about 4 mM. The reaction mixture may also contain about 10 mM B-mercaptoethanol and 0.05–1 mg/mL bovine serum albumin. An especially preferred buffer is WB4 buffer (67 mM Tris buffer pH 8.8, 4 mM MgCl₂, 16 mM (NH₄)₂SO₄, 10 mM β-mercaptoethanol and 0.1 mg/mL albumin. Preferred conditions for performing the reaction are provided below in Example 3.

To conduct the PCR reaction, a mixture comprising the oligonucleotide primers, the four deoxynucleotides, a suitable buffer, the DNA to be amplified, and a heat-stable DNA-dependent DNA polymerase is prepared. The mixture is then processed through temperature cycles for the annealing, elongating, and melting steps until the desired degree of amplification is achieved. The amount of DNA produced can be determined, for example, by staining with ethidium bromide, optionally after separating amplified fragments on an agarose gel.

A possible complication of the amplification reaction is dimerization and amplification of the oligonucleotide primers themselves. This can be easily detected as low molecular weight (<100 base pair) fragments on an agarose gel. Amplified primer can be removed by agarose or polyacrylamide gel separation. The amount of amplified dimer may be reduced by minor adjustments to the conditions of the reaction, particularly the temperature of the annealing step. It is also preferable to pre-mix the primers, the deoxynucleotides, and the buffer, and heat the mixture to 80 degrees before adding the DNA to be amplified.

Amplification reactions using any the oligonucleotides of this invention as primers yield polynucleotide fragments encoding a portion of a Glycoprotein B. These fragments can be characterized by a number of techniques known to a practitioner of ordinary skill in the art. Some non-limiting methods for characterizing a fragment are as follows:

In one method, a fragment may be sequenced according to any method of sequence determination known in the art, including the Maxam & Gilbert method, or the Sanger & Nicholson method. Alternatively, the fragment may be submitted to any of the commercial organizations that provide a polynucleotide sequencing service. The fragment may optionally be cloned and/or amplified before sequencing. The nucleotide sequence can be used to predict the amino acid sequence encoded by the fragment. Sequence data can be used for comparison with other sequenced Glycoprotein B's, either at the polynucleotide level or the amino acid level, to identify the species of herpes virus present in the original source material. Sequence data can also be used in modeling algorithms to predict antigenic regions or three-dimensional structure.

In a second method of characterizing, the size of the fragment can be determined by any suitable method, such as running on a polyacrylamide or agarose gel, or centrifuging through an appropriate density gradient. For example, for RFHV and KSHV, the fragment between NIVPA and TVNCB is about 319 bases. Hence, the length of the entire amplified fragment including primer binding regions is about 386 bases. The corresponding fragment of sHV1 contains an additional 6 base pairs. The sHV1 fragment can therefore be distinguished from that of RFHV or KSHV, for example, by running amplified polynucleotide fragments from each in neighboring lanes of a separating gel, or by running the sHV1 fragment beside suitable molecular weight standards. Polynucleotide fragments identical in size to that of RFHV and KSHV may be from the same or a related viral species. Fragments substantially different in size are more likely to be derived from a different herpes virus.

In a third method of characterizing, a fragment can be tested by attempting to hybridize it with an oligonucleotide probe. In a preferred example, a fragment is tested for relatedness to the Glycoprotein B encoding region of RFHV or KSHV. The test is conducted using a probe comprising a sequence of a Glycoprotein B encoding region, or its genetic complement. Suitable probes are polynucleotides comprising sequences from RFHV or KSHV, such as the Type 3 oligonucleotides listed in Table 7.

The length and nature of the probe and the hybridization conditions are selected depending on the objectives of the test. If the objective is to detect only polynucleotides from RFHV or KSHV, including minor strain variants, then hybridization is performed under conditions of high stringency. A sequence from the respective Glycoprotein B is used. Longer length sequences improve the specificity of the test and can be used under conditions of higher stringency. Preferably, the probe will comprise a Glycoprotein B sequence of at least about 30 nucleotides; more preferably, the sequence will be at least about 50 nucleotides; even more preferably, the sequence will be at least about 75 nucleotides in length.

If the objective is to detect polynucleotides that are closely related but not identical to RFHV or KSHV, such as in a screening test or a test to recruit previously undescribed viruses of the RFHV/KSHV subfamily, then different conditions are chosen. Sequences from RFHV or KSHV may be used, but a mixture of the two or a degenerate probe is generally preferred. The length of the sequence and the conditions of the hybridization reaction are selected to provide sufficient specificity to exclude unwanted sequences, but otherwise provide a maximum degree of cross-reactivity amongst potential targets. Suitable conditions can be predicted using the formulas given earlier, by calculating the T_m and then calculating the corresponding temperature for the maximum degree of mismatch to be tolerated. The suitability of the conditions can be tested empirically by testing the cross-reactivity of the probes with samples containing known target polynucleotides encoding herpes Glycoprotein B.

The minimum degree of complementarity required for a stable duplex to form under the conditions of the assay will determine what Glycoprotein B sequences will hybridize with the probe. Consider, for example, a target obtained from a human or non-human primate, amplified to produce a fragment corresponding to bases 36–354 of SEQ. ID NO:3, and then probed with the corresponding fragment of the KSHV polynucleotide. According to the data in Table 2, if the hybridization reaction is performed under conditions that require only about 50% identity for a stable duplex to form, the probe may hybridize with targets from any of the sequenced gamma herpes Glycoprotein B genes, including hEBV and sHV1. If the reaction is performed under conditions that require at least about 65% identity between probe and target, preferably at least about 67% identity, more preferably at least about 70% identity, and even more preferably at least about 75% identity for a stable duplex to form, the assay will detect a target polynucleotide from the RFHV/KSHV subfamily; i.e., either RHFV, KSHV, or a closely related herpes virus with a Glycoprotein B polynucleotide not yet sequenced. Even under hybridization conditions that required only about 50–55% identity for a stable duplex to form, a positive reaction would not indicate the presence of bHV4, cHV2, or mHV68, since these viruses are not believed to be capable of infecting primates.

It is possible to combine characterization by size and characterization by hybridization. For example, the ampli-

fied polynucleotide may be separated on a gel of acrylamide or agarose, blotted to a membrane of suitable material, such as nitrocellulose, and then hybridized with a probe with a suitable label, such as ³²P. The presence of the label after washing reflects the presence of hybridizable material in the sample, while the migration distance compared with appropriate molecular weight standards reflects the size of the material. A fragment sequence hybridizing with one of the aforementioned probes under conditions of high stringency but having an unexpected size would indicate a Glycoprotein B sequence with a high degree of identity to the probe, but distinct from either RFHV or KSHV.

Use of polynucleotides and oligonucleotides to detect herpes virus infection

Polynucleotides encoding herpes virus Glycoprotein B, and synthetic oligonucleotides based thereupon, as embodied in this invention, are useful in the diagnosis of clinical conditions associated with herpes virus infection. For example, the presence of detectable herpes Glycoprotein B in a clinical sample may suggest that the respective herpes virus participated as an etiologic agent in the development of the condition. The presence of viral Glycoprotein B in a particular tissue, but not in surrounding tissue, may be useful in the localization of an infected lesion. Differentiating between gamma herpes virus and other herpes viruses in clinical samples may be useful in predicting the clinical course of an infection or selecting a drug suitable for treatment. Since Glycoprotein B is expressed by replicative virus, L-particles, and infected cells, we predict that it will serve as a useful marker for active and quiescent stages of the disease that involve expression of the protein in any of these forms.

The procedures for conducting diagnostic tests are extensively known in the art, and are routine for a practitioner of ordinary skill. Generally, to perform a diagnostic method of this invention, one of the compositions of this invention is provided as a reagent to detect a target in a clinical sample with which it reacts. For example, a polynucleotide of this invention may be used as a reagent to detect a DNA or RNA target, such as might be present in a cell infected with a herpes virus. A polypeptide of this invention may be used as a reagent to detect a target with which it is capable of forming a specific complex, such as an antibody molecule or (if the polypeptide is a receptor) the corresponding ligand. An antibody of this invention may be used as a reagent to detect a target it specifically recognizes, such as a polypeptide expressed by virally infected cells.

The target is supplied by obtaining a suitable tissue sample from an individual for whom the diagnostic parameter is to be measured. Relevant test samples are those obtained from individuals suspected of harboring a herpes virus. Many types of samples are suitable for this purpose, including those that are obtained near the suspected site of infection or pathology by biopsy or surgical dissection, in vitro cultures of cells derived therefrom, solubilized extracts, blood, and blood components. If desired, the target may be partially purified from the sample or amplified before the assay is conducted. The reaction is performed by contacting the reagent with the sample under conditions that will allow a complex to form between the reagent and the target. The reaction may be performed in solution, or on a solid tissue sample, for example, using histology sections. The formation of the complex is detected by a number of techniques known in the art. For example, the reagent may be supplied with a label and unreacted reagent may be removed from the complex; the amount of remaining label thereby indicating the amount of complex formed. Further

details and alternatives for complex detection are provided in the descriptions that follow.

To determine whether the amount of complex formed is representative of herpes infected or uninfected cells, the assay result is preferably compared with a similar assay conducted on a control sample. It is generally preferable to use a control sample which is from an uninfected source, and otherwise similar in composition to the clinical sample being tested. However, any control sample may be suitable provided the relative amount of target in the control is known or can be used for comparative purposes. It is often preferable to conduct the assay on the test sample and the control sample simultaneously. However, if the amount of complex formed is quantifiable and sufficiently consistent, it is acceptable to assay the test sample and control sample on different days or in different laboratories.

Accordingly, polynucleotides encoding Glycoprotein B of the RFHV/KSHV subfamily, and the synthetic oligonucleotides embodied in this invention, can be used to detect gamma herpes virus polynucleotide that may be present in a biological sample. General methods for using polynucleotides in specific diagnostic assays are well known in the art: see, e.g., Patent Application JP 5309000 (Iatron).

An assay employing a polynucleotide reagent may be rendered specific, for example: 1) by performing a hybridization reaction with a specific probe; 2) by performing an amplification with a specific primer, or 3) by a combination of the two.

To perform an assay that is specific due to hybridization with a specific probe, a polynucleotide is chosen with the required degree of complementarity for the intended target. Preferred probes include polynucleotides of at least about 16 nucleotides in length encoding a portion of the Glycoprotein B of RFHV, KSHV, or another member of the RFHV/KSHV subfamily. Increasingly preferred are probes comprising at least about 18, 21, 25, 30, 50, or 100 nucleotides of the Glycoprotein B encoding region. Also preferred are degenerate probes capable of forming stable duplexes with polynucleotides of the RFHV/KSHV subfamily under the conditions used, but not polynucleotides of other herpes viruses.

The probe is generally provided with a label. Some of the labels often used in this type of assay include radioisotopes such as ³²P and ³³P, chemiluminescent or fluorescent reagents such as fluorescein, and enzymes such as alkaline phosphatase that are capable of producing a colored solute or precipitant. The label may be intrinsic to the reagent, it may be attached by direct chemical linkage, or it may be connected through a series of intermediate reactive molecules, such as a biotin-avidin complex, or a series of inter-reactive polynucleotides. The label may be added to the reagent before hybridization with the target polynucleotide, or afterwards. To improve the sensitivity of the assay, it is often desirable to increase the signal ensuing from hybridization. This can be accomplished by using a combination of serially hybridizing polynucleotides or branched polynucleotides in such a way that multiple label components become incorporated into each complex. See U.S. Pat. No. 5,124,246 (Urdea et al.).

If desired, the target polynucleotide may be extracted from the sample, and may also be partially purified. To measure viral particles, the preparation is preferably enriched for DNA; to measure active transcription of Glycoprotein B, the preparation is preferably enriched for RNA. Generally, it is anticipated that the level of polynucleotide of a herpes virus will be low in clinical samples: there may be just a few copies of DNA encoding the Glycoprotein B per cell where the virus is latent, or up to several hundred copies

of DNA per cell where the virus is replicating. The level of mRNA will be higher in cells where the protein is actively expressed than those where the gene is inactive. It may therefore be desirable to enhance the level of target in the sample by amplifying the DNA or RNA. A suitable method of amplification is a PCR, which is preferably conducted using one or more of the oligonucleotide primers embodied in this invention. RNA may be amplified by making a cDNA copy using a reverse transcriptase, and then conducting a PCR using the aforementioned primers.

The target polynucleotide can be optionally subjected to any combination of additional treatments, including digestion with restriction endonucleases, size separation, for example by electrophoresis in agarose or polyacrylamide, and affixation to a reaction matrix, such as a blotting material.

Hybridization is allowed to occur by mixing the reagent polynucleotide with a sample suspected of containing a target polynucleotide under appropriate reaction conditions. This may be followed by washing or separation to remove unreacted reagent. Generally, both the target polynucleotide and the reagent must be at least partly equilibrated into the single-stranded form in order for complementary sequences to hybridize efficiently. Thus, it may be useful (particularly in tests for DNA) to prepare the sample by standard denaturation techniques known in the art.

The level of stringency chosen for the hybridization conditions depends on the objective of the test. If it is desired that the test be specific for RFHV or KSHV, then a probe comprising a segment of the respective Glycoprotein B is used, and the reaction is conducted under conditions of high stringency. For example, a preferred set of conditions for use with a preferred probe of 50 nucleotides or more is 6xSSC at 37° C. in 50% formamide, followed by a wash at low ionic strength. This will generally require the target to be at least about 90% identical with the polynucleotide probe for a stable duplex to form. The specificity of the reaction for the particular virus in question can also be increased by increasing the length of the probe used. Thus, longer probes are particularly preferred for this application of the invention. Alternatively, if it is desired that the test also be able to detect other herpes viruses related to KSHV, then a lower stringency is used. Suitable probes include fragments from the KSHV Glycoprotein B polynucleotide, a mixture thereof, or oligonucleotides such as those listed in Table 7.

Appropriate hybridization conditions are determined to permit hybridization of the probe only to Glycoprotein B sequences that have the desired degree of identity with the probe. The stringency required depends on the length of the polynucleotide probe, and the degree of identity between the probe and the desired target sequence. Consider, for example, a probe consisting of the KSHV polynucleotide fragment between the hybridization sites of NIVPA and TVNCB. Conditions requiring a minimum identity of 60% would result in a stable duplex formed with a corresponding polynucleotide of KSHV and other gamma herpes viruses such as sHV1; conditions requiring a minimum identity of 90% would result in a stable duplex forming only with a polynucleotide from KSHV and closely related variants. Conditions of intermediate stringency requiring a minimum identity of 65–70% would permit duplexes to form with a Glycoprotein B polynucleotide of KSHV, and some other members of the RFHV/KSHV subfamily, but not with corresponding polynucleotides of other known herpes viruses, including gamma herpes viruses eHV2, sHV1, mHV68, bHV4, EBV, and other human pathogens such as hCMV, hHV6, hVZV, and HSV1.

Conditions can be estimated beforehand using the formula given earlier. Preferably, the exact conditions are confirmed by testing the probe with separate samples known to contain polynucleotides, both those desired to be detected and those desired to go undetected in the assay. Such samples may be provided either by synthesizing the polynucleotides from published sequences, or by extracting and amplifying DNA from tissues believed to be infected with the respective herpes virus. Determining hybridization conditions is a matter of routine adjustment for a practitioner of ordinary skill, and does not require undue experimentation. Since eHV2, sHV1, mHV68, bHV4 and EBV are more closely identical to the RFHV/KSHV subfamily than alpha and beta herpes viruses, conditions that exclude gamma herpes viruses outside the RFHV/KSHV subfamily will generally also exclude the other herpes viruses listed in Table 1. In addition, if it is believed that certain viruses will not be present in the sample to be tested in the ultimate determination (such as eHV2, mHV68 or bHV4 in a human tissue sample), then the corresponding target sequences may optionally be omitted when working out the conditions of the assay. Thus, conditions can be determined that would permit Type 2 oligonucleotide probes such as those listed in Table 6 to form a stable duplex both with polypeptides comprising SEQ. ID NO: 1 or SEQ. ID NO:3, but not a sequence selected from the group consisting of SEQ. ID NO:5–13. Conditions can also be determined that would permit a suitable fragment comprising at least 21 or more consecutive bases of SEQ. ID NO: 1 or SEQ. ID NO:3 to form a stable duplex both with a polynucleotide comprising SEQ. ID NO:1 and SEQ. ID NO:3, but not a polynucleotide comprising any one of SEQ. ID NO:5–13.

Alternatively, to conduct an assay that is specific due to amplification with a specific primer: DNA or RNA is prepared from the biological sample as before. Optionally, the target polynucleotide is pre-amplified in a PCR using primers which are not species specific, such as those listed in Table 4 or 6. The target is then amplified using specific primers, such as those listed in Table 7, or a combination of primers from Table 4, 6, and 7. In a preferred embodiment, two rounds of amplification are performed, using oligonucleotide primers in a nested fashion: virus-specific or non-specific in the first round; virus-specific in the second round. This provides an assay which is both sensitive and specific.

Use of a specific Type 3 primer during amplification is sufficient to provide the required specificity. A positive test may be indicated by the presence of sufficient reaction product at the end of the amplification series. Amplified polynucleotide can be detected, for example, by blotting the reaction mixture onto a medium such as nitrocellulose and staining with ethidium bromide. Alternatively, a radiolabeled substrate may be added to the mixture during a final amplification cycle; the incorporated label may be separated from unincorporated label (e.g., by blotting or by size separation), and the label may be detected (e.g. by counting or by autoradiography). If run on a gel of agarose or polyacrylamide, the size of the product may help confirm the identity of the amplified fragment. Specific amplification can also be followed by specific hybridization, by using the amplification mixture obtained from the foregoing procedure as a target source for the hybridization reaction outlined earlier.

Use of polynucleotides for gene therapy

Embodied in this invention are pharmaceuticals comprising virus-specific polynucleotides, polypeptides, or antibodies as an active ingredient. Such compositions may decrease

the pathology of the virus or infected cells on their own, or render the virus or infected cells more susceptible to treatment by non-specific pharmaceutical compounds.

Polynucleotides of this invention encoding part of a herpes virus Glycoprotein B may be used, for example, for administration to an infected individual for purposes of gene therapy (see generally U.S. Pat. No. 5,399,346: Anderson et al.). The general principle is to administer the polynucleotide in such a way that it either promotes or attenuates the expression of the polypeptide encoded therein.

A preferred mode of gene therapy is to provide the polynucleotide in such a way that it will be replicated inside the cell, enhancing and prolonging the effect. Thus, the polynucleotide is operatively linked to a suitable promoter, such as the natural promoter of the corresponding gene, a heterologous promoter that is intrinsically active in cells of the target tissue type, or a heterologous promoter that can be induced by a suitable agent. Entry of the polynucleotide into the cell is facilitated by suitable techniques known in the art, such as providing the polynucleotide in the form of a suitable vector, such as a viral expression vector, or encapsulation of the polynucleotide in a liposome. The polynucleotide may be injected systemically, or provided to the site of infection by an antigen-specific homing mechanism, or by direct injection.

In one variation, the polynucleotide comprises a promoter linked to the polynucleotide strand with the same orientation as the strand that is transcribed during the course of a herpes virus infection. Preferably, the Glycoprotein B that is encoded includes an external component, a transmembrane component, and signal sequences for transport to the surface. Virally infected cells transfected with polynucleotides of this kind are expected to express an enhanced level of Glycoprotein B at the surface. Enhancing Glycoprotein B expression in this fashion may enhance recognition of these cells by elements of the immune system, including antibody (and antibody-dependent effectors such as ADCC), and virus-specific cytotoxic T cells.

In another variation, the polynucleotide comprises a promoter linked to the polynucleotide strand with the opposite orientation as the strand that is transcribed during the course of a herpes virus infection. Virally infected cells transfected with polynucleotides of this kind are expected to express a decreased level of Glycoprotein B. The transcript is expected to hybridize with the complementary strand transcribed by the viral gene, and prevent it from being translated. This approach is known as anti-sense therapy. RFHV/KSHV subfamily polypeptides with Glycoprotein B activity and fragments thereof

The RFHV and KSHV polynucleotide sequences shown in FIG. 1 have open reading frames. The polypeptide encoded thereby are shown in SEQ. ID NO:2 and SEQ. ID NO:4, respectively. Encoded between the hybridizing regions of the primers NIVPA and TVNCB used to obtain the polynucleotide sequence is a 106 amino acid fragment of the Glycoprotein B molecule which is 91% identical between RFHV and KSHV. The full protein sequence of KSHV Glycoprotein B is shown in SEQ. ID NO:94. A Glycoprotein B fragment of a third member of the RFHV/KSHV subfamily, RFHV2, is shown in SEQ. ID NO:97.

There are a number of homologous residues to Glycoprotein B molecules of other sequenced herpes viruses. The longest sequence contained in SEQ. ID NO:2 or SEQ. ID NO:4 but not in the known sequences of other herpes viruses is 9 amino acids in length, with two exceptions (SEQ. ID NOS:64 and 65). Longer matching portions are found elsewhere in the Glycoprotein B amino acid sequence. The

longest is the 21 amino acid sequence from bHV4 shown in SEQ. ID NO:99; the rest are all 16 amino acids long or less. Other than SEQ. ID NO:99 exception, any fragment of the RFHV and KSHV Glycoprotein B protein sequence that is 17 amino acids or longer is believed to be specific for RFHV or KSHV, respectively, or to closely related strains. Since bHV4 and the other viruses with matching segments are not believed to be capable of infecting primates, any fragment of about 10 amino acids or more found in a primate that was contained in SEQ. ID NO:4 would indicate the presence of an infectious agent closely related to KSHV.

This invention embodies both intact Glycoprotein B from herpes viruses of the RFHV/KSHV subfamily, and any fragment thereof that is specific for the subfamily. Preferred Glycoprotein B fragments of this invention are at least 10 amino acids in length; more preferably they are at least 13 amino acids in length; more preferably they are at least 17 amino acids in length; more preferably they are at least about 20 amino acids in length; even more preferably they are at least about 25 amino acids in length, still more preferably they are at least about 30 amino acids in length.

The amino acid sequence of the RFHV and KSHV Glycoprotein B fragment shown in SEQ. ID NOS:2, 4, 94 and 96 can be used to identify virus-specific and cross-reactive antigenic regions.

In principle, a specific antibody could recognize any amino acid difference between sequences that is not also shared by the species from which the antibody is derived. Antibody binding sites are generally big enough to encompass 5–9 amino acid residues of an antigen, and are quite capable of recognizing a single amino acid difference. Specific antibodies may be part of a polyclonal response arising spontaneously in animals infected with a virus expressing the Glycoprotein B. Specific antibodies may also be induced by injecting an experimental animal with either the intact Glycoprotein B or a Glycoprotein B fragment.

Thus, any peptide of 5 amino acids or more that is unique to KSHV is a potential virus-specific antigen, and could be recognized by a KSHV-specific antibody. Similarly, any peptide of sufficient length shared within the RFHV/KSHV subfamily but not with other herpes viruses is a potential subfamily-specific antigen.

Some examples of preferred peptides are shown in Table 8. Practitioners in the art will immediately recognize that other peptides with similar specificities may be designed by minor alterations to the length of the peptides listed and/or moving the frame of the peptide a few residues in either direction.

The Class I peptides shown in Table 8 are conserved between Glycoprotein B of KSHV and that of certain other members of the gamma herpes virus subfamily. An antibody directed against one such Glycoprotein B in this region may therefore cross-react with some of the others. Class II peptides are conserved between Glycoprotein B of RFHV and KSHV, but not with other gamma herpes viruses. An antibody directed against this region is expected to cross-react between RFHV, KSHV, and other viruses of the RFHV/KSHV subfamily; but not with herpes viruses outside the subfamily. Class III peptides are different between Glycoprotein B of RFHV, KSHV, and other known gamma herpes viruses. An antibody binding to this region, particularly to non-identical residues contained therein, is expected to distinguish RFHV and KSHV Glycoprotein B from each other, and from Glycoprotein B of more distantly related herpes viruses.

TABLE 8

Antigen Peptides				
Specificity		Sequence	Length	SEQ. ID NO:
Class I:	Shared with bHV4	YRKATSVTVYRG	13	64
	bHV4, mHV68	RYFSQP	6	66
	Shared amongst RFHV/KSHV subfamily and some other gamma herpes viruses	IYAEPGWFPGIYRVR	15	65
		IYAEPGWFPGIYRVRTTVNCE	21	99
	mHV68	VLEELSRAWCREQVRD	16	100
Class II:		VTVYRG	6	67
		AITNKYE	7	68
	Shared amongst RFHV/KSHV subfamily	SHMDSTY	7	69
		VENTFTD	7	70
		TVFLQPV	7	71
Class III:		TDNIQRY	7	72
	Specific for RFHV KSHV RFHV KSHV	RGMTAA	7	73
		RGITSA	7	75
		PVLYSEP	7	74
		PVIYAE	7	76

¹- Not shared with any other sequenced herpes virus; may be present in some unsequenced RFHV/KSHV subfamily viruses

Particularly preferred peptides from Class III are those encompassing regions of Glycoprotein B with the polarity characteristics appropriate for an antigen epitope, as described in the Example section. Given the complete sequence of a Glycoprotein B from KSHV and other members of the RFHV/KSHV subfamily, virus- or subfamily-specific peptides can be predicted for other regions of the molecule by a similar analysis.

Preparation of polypeptides

Polypeptides of this invention may be prepared by several different methods, all of which will be known to a practitioner of ordinary skill.

For example, short polypeptides of about 5–50 amino acids in length are conveniently prepared from sequence data by chemical synthesis. A preferred method is the solid-phase Merrifield technique. Alternatively, a messenger RNA encoding the desired polypeptide may be isolated or synthesized according to one of the methods described earlier, and translated using an in vitro translation system, such as the rabbit reticulocyte system. See, e.g., Dorsky et al.

Longer polypeptides, up to and including the entire Glycoprotein B, are conveniently prepared using a suitable expression system. For example, the encoding strand of a full-length cDNA can be operatively linked to a suitable promoter, inserted into an expression vector, and transfected into a suitable host cell. The host cell is then cultured under conditions that allow transcription and translation to occur, and the polypeptide is subsequently recovered. For examples of the expression and recovery of Glycoprotein B from other species of herpes virus, see, for example, U.S. Pat. No. 4,642,333 (Person); U.S. Pat. No. 5,244,792 (Burke et al.); Manservigi et al.

For many purposes, it is particularly convenient to use a recombinant Glycoprotein B polynucleotide that includes the regions encoding signals for transport to the cell surface, but lacks the region encoding the transmembrane domain of the protein. The polynucleotide may be truncated 5' to the transmembrane encoding region, or it may comprise both extracellular and cytoplasmic encoding region but lack the transmembrane region. Constructs of this nature are expected to be secreted from the cell in a soluble form.

Where it is desirable to have a Glycoprotein B fragment that is a monomer, the recombinant may be designed to limit translation to about the first 475 amino acids of the protein.

For example, to express any of these forms of Glycoprotein B in yeast, a cassette may be prepared using the glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) promoter region and terminator region. GAPDH gene fragments are identified in a yeast library, isolated and ligated in the appropriate configuration. The cassette is cloned into pBR322, isolated and confirmed by DNA sequencing. A pCI/1 plasmid is constructed containing a Glycoprotein B insert and GAPDH promoter and terminator regions. The plasmid is used to transform yeast strain *S. cerevisiae*. After culture, the yeast cells are pelleted by centrifugation, resuspended in a buffer containing protease inhibitors such as 1 mM phenylmethylsulfonyl fluoride and 0.1 μ g/ml pepstatin. The washed cells are disrupted by vortexing with glass beads and recentrifuged. The presence in the supernatant of a Glycoprotein B of the correct size may be confirmed, for example, by Western blot using an antibody against Glycoprotein B, prepared as described in a following section. Glycoprotein B may be purified from the supernatant by a combination of standard protein chemistry techniques, including ion exchange chromatography, affinity chromatography using antibody or substrate, and high-pressure liquid chromatography.

To express Glycoprotein B in mammalian cells, for example, a mammalian expression vector such as pSV1/dhfr may be used. This has an ampicillin-resistance beta-lactamase gene, and a selectable mammalian cell marker, dihydrofolate reductase linked to the SV40 early promoter. Glycoprotein B polynucleotide blunt-end fragments are ligated into the pSV1/dhfr vector and digested with endonucleases to provide a cassette including the SV40 promoter, the Glycoprotein encoding region, and the SV40 splice and polyadenylation sites. The plasmids are used, for example, to transform CHO cells deficient in dhfr, and transfectants are selected. Cells expressing Glycoprotein B may be identified, for example, by immunofluorescence using anti-Glycoprotein B as the primary antibody.

In another example, recombinant plasmids for expressing Glycoprotein B are cloned under control of the Rous sar-

coma virus long terminal repeat in the episomal replicating vector pRP-RSV. This plasmid contains the origin of replication and early region of the human papovavirus BK, as well as the dhfr resistance marker. The vector can then be used, for example, to transform human 293 cells. By using a Glycoprotein B encoding region devoid of the transmembrane spanning domain, the Glycoprotein B polypeptide is constitutively secreted into the culture medium at 0.15–0.25 pg/cell/day. In the presence of 0.6–6 μ M methotrexate, production may be increased 10- to 100-fold, because of an amplification of the episomal recombinant. Glycoprotein B prepared in this way are appropriate, *inter alia*, for use in diagnosis, and to prepare vaccines protective against new and recurrent herpes virus infections (Manservigi et al).

Use of polypeptides to assess herpes virus infection

The polypeptides embodied in this invention may be used to detect or assess the status of a herpes virus infection in an individual in several different applications.

In one application, a polypeptide encoding a portion of a herpes virus Glycoprotein B is supplied as a reagent for an assay to detect the presence of antibodies that can specifically recognize it. Such antibodies may be present, for example, in the circulation of an individual with current or past herpes virus exposure.

The presence of antibodies to Glycoprotein B in the circulation may provide a sensitive and early indication of viral infection. Since Glycoprotein B is a functional component of the viral envelope, it is produced in greater quantity than other transcripts sequestered within the viral particle. Its distribution is wider than transcripts that appear only transiently in the life cycle of the virus. Furthermore, it may be expressed not only by intact virus, but also by non-infective products of virally infective cells, such as L-particles. Glycoprotein B from various species of herpes virus are known to be strongly immunogenic. Thus, detection of antibody to Glycoprotein B in an individual may be an indication of ongoing active herpes virus infection, latent infection, previous exposure, or treatment with a Glycoprotein B vaccine.

Suitable clinical samples in which to measure antibody levels include serum or plasma from an individual suspected of having a gamma herpes virus infection. The presence of the antibody is determined, for example, by an immunoassay.

A number of immunoassay methods are established in the art for performing the quantitation of antibody using viral peptides (see, e.g., U.S. Pat. No. 5,350,671: Houghton et al.). For example, the test sample potentially containing the specific antibody may be mixed with a pre-determined non-limiting amount of the reagent polypeptide. The reagent may contain a directly attached label, such as an enzyme or a radioisotope. For a liquid-phase assay, unreacted reagents are removed by a separation technique, such as filtration or chromatography. Alternatively, the antibody in the sample may be first captured by a reagent on a solid phase. This may be, for example, the specific polypeptide, an anti-immunoglobulin, or Protein A. The captured antibody is then detected with a second reagent, such as the specific polypeptide, anti-immunoglobulin, or protein A with an attached label. At least one of the capture reagent or the detecting reagent must be the specific polypeptide. In a third variation, cells or tissue sections containing the polypeptide may be overlaid first with the test sample containing the antibody, and then with a detecting reagent such as labeled anti-immunoglobulin. In all these examples, the amount of label captured in the complex is positively related to the amount of specific antibody present in the test sample.

Similar assays can be designed in which antibody in the test sample competes with labeled antibody for binding to a limiting amount of the specific peptide. The amount of label in the complex is then negatively correlated with the amount of specific antibody in the test sample. Results obtained using any of these assays are compared between test samples, and control samples from an uninfected source.

By selecting the reagent polypeptide appropriately, antibodies of a desired specificity may be detected. For example, if the intact Glycoprotein B is used, or a fragment comprising regions that are conserved between herpes virus, then antibodies detected in the test samples may be virus specific, cross-reactive, or both. A multi-epitope reagent is preferred for a general screening assay for antibodies related to herpes virus infection. To render the assay specific for antibodies directed either against RFHV or against KSHV, antigen peptides comprising non-conserved regions of the appropriate Glycoprotein B molecule are selected, such as those listed in Class III of Table 8. Preferably, a mixture of such peptides is used. To simultaneously detect antibodies against RFHV, KSHV, and closely related viruses of the gamma herpes family, but not SHV1 and EBV, antigen peptides are selected with the properties of those listed in Class II of Table 8. Preferably, a mixture of such peptides is used.

Antibodies stimulated during a herpes virus infection may subside once the infection resolves, or they may persist as part of the immunological memory of the host. In the latter instance, antibodies due to current infection may be distinguished from antibodies due to immunological memory by determining the class of the antibody. For example, an assay may be conducted in which antibody in the test sample is captured with the specific polypeptide, and then developed with labeled anti-IgM or anti-IgG. The presence of specific antibody in the test sample of the IgM class indicates ongoing infection, while the presence of IgG antibodies alone indicates that the activity is due to immunological memory of a previous infection or vaccination.

Use of polypeptides to design or screen anti-viral drugs

Interfering with the Glycoprotein B gene or gene product would modify the infection process, or the progress of this disease. It is an objective of this invention to provide a method by which useful pharmaceutical compositions and methods of employing such compounds in the treatment of gamma herpes virus infection can be developed and tested. Particularly preferred are pharmaceutical compounds useful in treating infections by RFHV, KSHV and other members of the RFHV/KSHV subfamily. Suitable drugs are those that interfere with transcription or translation of the Glycoprotein B gene, and those that interfere with the biological function of the polypeptide encoded by the gene. It is not necessary that the mechanism of interference be known; only that the interference be preferential for reactions associated with the infectious process.

Preferred drugs include those that competitively interfere with the binding of the Glycoprotein B to its substrate on target cells, such as heparan sulfate and its analogs. Also preferred are drugs that competitively interfere with any interaction of Glycoprotein B to other viral envelope components that may be necessary for the virus to exert one of its biologic functions, such as penetration of target cells. Also preferred are molecules capable of cross-linking or otherwise immobilizing the Glycoprotein B, thereby preventing it from binding its substrate or performing any biological function that plays a role in viral infectivity.

This invention provides methods for screening pharmaceutical candidates to determine which are suitable for clinical use. The methods may be brought to bear on

antiviral compounds that are currently known, and those which may be designed in the future.

The method involves combining an active Glycoprotein B with the pharmaceutical candidate, and determining whether the biochemical function is altered by the pharmaceutical candidate. The Glycoprotein B may be any fragment encoded by the Glycoprotein B gene of the RFHV/KSHV subfamily that has Glycoprotein B activity. Suitable fragments may be obtained by expressing a genetically engineered polypeptide encoding an active site of the molecule, or by cleaving the Glycoprotein B with proteases and purifying the active fragments. In a preferred embodiment, the entire Glycoprotein B is provided. The reaction mixture will also comprise other components necessary to measure the biological activity of the protein. For example, in an assay to measure substrate binding, heparan sulfate or an analog thereof may be provided, perhaps linked to a solid support to facilitate measurement of the binding reaction.

One embodiment of the screening method is to measure binding of the pharmaceutical candidate directly to the isolated Glycoprotein B, or a fragment thereof. Compounds that bind to an active site of the molecule are expected to interfere with Glycoprotein B activity. Thus, the entire Glycoprotein B, or a fragment comprising the active site, is mixed with the pharmaceutical candidate. Binding of the candidate can be measured directly, for example, by providing the candidate in a radiolabeled or stable-isotope labeled form. The presence of label bound to the Glycoprotein B can be determined, for example, by precipitating the Glycoprotein B with a suitable antibody, or by providing the molecule attached to a solid phase, and washing the solid phase after the reaction. Binding of the candidate to the Glycoprotein B may also be observed as a conformational change, detected for example by difference spectroscopy, nuclear magnetic resonance, or circular dichroism. Alternatively, binding may be determined in a competitive assay: for example, Glycoprotein B is mixed with the candidate, and then labeled nucleotide or a fragment of a regulatory subunit is added later. Binding of the candidate to the biochemically relevant site should inhibit subsequent binding of the labeled compound.

A second embodiment of the screening method is to measure the ability of the pharmaceutical candidate to inhibit the binding of Glycoprotein B to a substrate or substrate analog. A preferred analog is heparin, coupled to a solid support such as Sepharose™ beads. Inhibition may be measured, for example, by providing a radiolabel to the Glycoprotein B, incubating it with the pharmaceutical candidate, adding the affinity resin, then washing and counting the resin to determine if the candidate has decreased the amount of radioactivity bound. Pharmaceutical candidates may also be tested for their ability to competitively interfere with interactions between Glycoprotein B and other herpes virus proteins.

A third embodiment of the screening method is to measure the ability of the pharmaceutical candidate to inhibit an activity of an active particle, such as a viral particle, mediated by Glycoprotein B. A particle is engineered to express Glycoprotein B, but not other components that are capable of mediating the same function. The ability of the particle to exhibit a biological function, such as substrate binding or membrane fusion, is then measured in the presence and absence of the pharmaceutical candidate by providing an appropriate target.

This invention also provides for the development of pharmaceuticals for the treatment of herpes infection by rational drug design. See, generally, Hodgson, and Erickson

et al. In this embodiment, the three-dimensional structure of the Glycoprotein B is determined, either by predictive modeling based on the amino acid sequence, or preferably, by experimental determination. Experimental methods include antibody mapping, mutational analysis, and the formation of anti-idiotypes. Especially preferred is X-ray crystallography. Knowing the three-dimensional structure of the glycoprotein, especially the orientation of important amino acid groups near the substrate binding site, a compound is designed de novo, or an existing compound is suitably modified. The designed compound will have an appropriate charge balance, hydrophobicity, and/or shape to permit it to attach near an active site of the Glycoprotein B, and sterically interfere with the normal biochemical function of that site. Preferably, compounds designed by this method are subsequently tested in a drug screening assay, such as those outlined above.

Antibodies against Glycoprotein B and their preparation

The amino acid sequence of the Glycoprotein B molecules embodied herein are foreign to the hosts they infect. Glycoprotein B from other species of herpes virus are known to be strongly immunogenic in mammals. Anti-Glycoprotein B is formed in humans, for example, as a usual consequence of infection with hCMV. By analogy, it is expected that Glycoprotein B of RFHV, KSHV, and other members of the RFHV/KSHV subfamily will be immunogenic in mammals, including humans. These expectations are supported by the observations described in the Example section below.

Antibodies against a polypeptide are generally prepared by any method known in the art. To stimulate antibody production in an animal experimentally, it is often preferable to enhance the immunogenicity of a polypeptide by such techniques as polymerization with glutaraldehyde, or combining with an adjuvant, such as Freund's adjuvant. The immunogen is injected into a suitable experimental animal: preferably a rodent for the preparation of monoclonal antibodies; preferably a larger animal such as a rabbit or sheep for preparation of polyclonal antibodies. It is preferable to provide a second or booster injection after about 4 weeks, and begin harvesting the antibody source no less than about 1 week later.

Sera harvested from the immunized animals provide a source of polyclonal antibodies. Detailed procedures for purifying specific antibody activity from a source material are known within the art. If desired, the specific antibody activity can be further purified by such techniques as protein A chromatography, ammonium sulfate precipitation, ion exchange chromatography, high-performance liquid chromatography and immunoaffinity chromatography on a column of the immunizing polypeptide coupled to a solid support.

Polyclonal antibodies raised by immunizing with an intact Glycoprotein B or a fragment comprising conserved sequences may be cross-reactive between herpes viruses. Antibodies that are virus or subfamily specific may be raised by immunizing with a suitably specific antigen, such as those listed above in Table 8. Alternatively, polyclonal antibodies raised against a larger fragment may be rendered specific by removing unwanted activity against other virus Glycoprotein B's, for example, by passing the antibodies over an adsorbent made from Glycoprotein B and collecting the unbound fraction.

Alternatively, immune cells such as splenocytes can be recovered from the immunized animals and used to prepare a monoclonal antibody-producing cell line. See, for example, Harrow & Lane (1988), U.S. Pat. No. 4,472,500 (Milstein et al.), and U.S. Pat. No. 4,444,887 (Hoffman et al.).

Briefly, an antibody-producing line can be produced *inter alia* by cell fusion, or by transforming antibody-producing cells with Epstein Barr Virus, or transforming with oncogenic DNA. The treated cells are cloned and cultured, and clones are selected that produce antibody of the desired specificity. Specificity testing can be performed on culture supernatants by a number of techniques, such as using the immunizing polypeptide as the detecting reagent in a standard immunoassay, or using cells expressing the polypeptide in immunohistochemistry. A supply of monoclonal antibody from the selected clones can be purified from a large volume of tissue culture supernatant, or from the ascites fluid of suitably prepared host animals injected with the clone.

Effective variations of this method include those in which the immunization with the polypeptide is performed on isolated cells. Antibody fragments and other derivatives can be prepared by methods of standard protein chemistry, such as subjecting the antibody to cleavage with a proteolytic enzyme. Genetically engineered variants of the antibody can be produced by obtaining a polynucleotide encoding the antibody, and applying the general methods of molecular biology to introduce mutations and translate the variant.

Monoclonal antibodies raised by injecting an intact Glycoprotein B or a fragment comprising conserved sequences may be cross-reactive between herpes viruses. Antibodies that are virus or subfamily specific may be raised by immunizing with a suitably specific antigen, as may be selected from Table 8. Alternatively, virus-specific clones may be selected from the cloned hybridomas by using a suitable antigen, such as one selected from Class III of Table 8, in the screening process.

Specific antibodies against herpes virus Glycoprotein B have a number of uses in developmental, diagnostic and therapeutic work. For example, antibodies can be used in drug screening (see U.S. Pat. No. 5,120,639). They may also be used as a component of a passive vaccine, or for detecting herpes virus in a biological sample and for drug targeting, as described in the following sections.

Anti-idiotypes relating to Glycoprotein B may also be prepared. This is accomplished by first preparing a Glycoprotein B antibody, usually a monoclonal antibody, according to the aforementioned methodology. The antibody is then used as an immunogen in a volunteer or experimental animal to raise an anti-idiotypic. The anti-idiotypic may be either monoclonal or polyclonal, and its development is generally according to the methodology used for the first antibody. Selection of the anti-idiotypic or hybridoma clones expressing anti-idiotypic is done using the immunogen antibody as a positive selector, and using antibodies of unrelated specificity as negative selectors. Usually, the negative selector antibodies will be a polyclonal immunoglobulin preparation or a pool comprising monoclonal immunoglobulins of the same immunoglobulin class and subclass, and the same species as the immunogen antibody. An anti-idiotypic may be used as an alternative component of an active vaccine against Glycoprotein B.

Use of antibodies for detecting Glycoprotein B in biological samples

Antibodies specific for Glycoprotein B can be used to detect Glycoprotein B polypeptides and fragments of viral origin that may be present, for example, in solid tissue samples and cultured cells. Immunohistological techniques to carry out such determinations will be obvious to a practitioner of ordinary skill. Generally, the tissue is preserved by a combination of techniques which may include freezing, exchanging into different solvents, fixing with agents such as paraformaldehyde, drying with agents such as

alcohol, or embedding in a commercially available medium such as paraffin or OCT. A section of the sample is suitably prepared and overlaid with a primary antibody specific for the protein.

The primary antibody may be provided directly with a suitable label. More frequently, the primary antibody is detected using one of a number of developing reagents which are easily produced or available commercially. Typically, these developing reagents are anti-immunoglobulin or protein A, and they typically bear labels which include, but are not limited to: fluorescent markers such as fluorescein, enzymes such as peroxidase that are capable of precipitating a suitable chemical compound, electron dense markers such as colloidal gold, or radioisotopes such as ^{125}I . The section is then visualized using an appropriate microscopic technique, and the level of labeling is compared between the suspected virally infected and a control cell, such as cells surrounding the area of infection or taken from a remote site.

Proteins encoded by a Glycoprotein B gene can also be detected in a standard quantitative immunoassay. If the protein is secreted or shed from infected cell in any appreciable amount, it may be detectable in plasma or serum samples. Alternatively, the target protein may be solubilized or extracted from a solid tissue sample. Before quantitating, the protein may optionally be affixed to a solid phase, such as by a blot technique or using a capture antibody.

A number of immunoassay methods are established in the art for performing the quantitation. For example, the protein may be mixed with a pre-determined non-limiting amount of the reagent antibody specific for the protein. The reagent antibody may contain a directly attached label, such as an enzyme or a radioisotope, or a second labeled reagent may be added, such as anti-immunoglobulin or protein A. For a solid-phase assay, unreacted reagents are removed by washing. For a liquid-phase assay, unreacted reagents are removed by some other separation technique, such as filtration or chromatography. The amount of label captured in the complex is positively related to the amount of target protein present in the test sample. A variation of this technique is a competitive assay, in which the target protein competes with a labeled analog for binding sites on the specific antibody. In this case, the amount of label captured is negatively related to the amount of target protein present in a test sample. Results obtained using any such assay are compared between test samples, and control samples from an uninfected source.

Use of antibodies for drug targeting

An example of how antibodies can be used in therapy of herpes virus infection is in the specific targeting of effector components. Virally infected cells generally display peptides of the virus, especially proteins expressed on the outside of the viral envelope. The peptide therefore provides a marker for infected cells that a specific antibody can bind to. An effector component attached to the antibody therefore becomes concentrated near the infected cells, improving the effect on those cells and decreasing the effect on uninfected cells. Furthermore, if the antibody is able to induce endocytosis, this will enhance entry of the effector into the cell interior.

For the purpose of targeting, an antibody specific for the viral polypeptide (in this case, a region of a Glycoprotein B) is conjugated with a suitable effector component, preferably by a covalent or high-affinity bond. Suitable effector components in such compositions include radionuclides such as ^{131}I , toxic chemicals, and toxic peptides such as diphtheria toxin. Another suitable effector component is an antisense polynucleotide, optionally encapsulated in a liposome.

Diagnostic kits

Diagnostic procedures using the polynucleotides, oligonucleotides, peptides, or antibodies of this invention may be performed by diagnostic laboratories, experimental laboratories, practitioners, or private individuals. This invention provides diagnostic kits which can be used in these settings. The presence of a herpes virus in the individual may be manifest in a clinical sample obtained from that individual as an alteration in the DNA, RNA, protein, or antibodies contained in the sample. An alteration in one of these components resulting from the presence of a herpes virus may take the form of an increase or decrease of the level of the component, or an alteration in the form of the component, compared with that in a sample from a healthy individual. The clinical sample is optionally pre-treated for enrichment of the target being tested for. The user then applies a reagent contained in the kit in order to detect the changed level or alteration in the diagnostic component.

Each kit necessarily comprises the reagent which renders the procedure specific: a reagent polynucleotide, used for detecting target DNA or RNA; a reagent antibody, used for detecting target protein; or a reagent polypeptide, used for detecting target antibody that may be present in a sample to be analyzed. The reagent is supplied in a solid form or liquid buffer that is suitable for inventory storage, and later for exchange or addition into the reaction medium when the test is performed. Suitable packaging is provided. The kit may optionally provide additional components that are useful in the procedure. These optional components include buffers, capture reagents, developing reagents, labels, reacting surfaces, means for detection, control samples, instructions, and interpretive information.

Other members of the RFHV/KSHV subfamily

RFHV and KSHV are exemplary members of the RFHV/KSHV subfamily. This invention embodies polynucleotide sequences encoding Glycoprotein B of other members of the subfamily, as defined herein. The consensus-degenerate gamma herpes virus oligonucleotide Type 1 and 2 primers, and the methods described herein are designed to be suitable for characterization of the corresponding polynucleotide fragment of other members of the RFHV/KSHV subfamily. One such member is another virus infecting monkeys, designated RFHV2. A segment of the Glycoprotein encoding sequence for this virus was cloned from RF tissue obtained from a *Macaca mulatta* monkey, as described in Example 12.

In order to identify and characterize other members of the family, reagents and methods of this invention are applied to DNA extracted from tissue samples suspected of being infected with such a virus.

Suitable sources of DNA for this purpose include biological samples obtained from a wide range of conditions occurring in humans and other vertebrates. Preferred are conditions in which the agent is suspected of being lymphotropic, similar to other members of the gamma herpes virus subfamily; for example, infectious mononucleosis of non-EBV origin. More preferred are conditions which resemble in at least one of their clinical or histological features the conditions with which RFHV or KSHV are associated. These include: a) conditions in which fibroproliferation is part of the pathology of the disease, especially in association with collagen deposition, and especially where the fibrous tissue is disorganized; b) conditions involving vascular dysplasia; c) conditions involving malignant transformation, especially but not limited to cells of lymphocyte lineage; d) conditions for which an underlying immunodeficiency contributes to the frequency or severity

of the disease; e) conditions which arise idiopathically at multiple sites in an organ or in the body as a whole; f) conditions which epidemiological data suggests are associated with an infectious or environmental agent. Conditions which fulfill more than one of these criteria are comparably more preferred. Some examples of especially preferred conditions include retroperitoneal fibrosis, nodular fibromatosis, pseudosarcomatous fibromatosis, fibrosarcomas, sclerosing mesenteritis, acute respiratory disease syndrome, idiopathic pulmonary fibrosis, diffuse proliferative glomerulonephritis of various types, gliomas, glioblastomas, gliosis, and all types of leukemias and lymphomas.

The type of tissue sample used will depend on the clinical manifestations of the condition. Samples more likely to contain a virus associated with the condition may be taken from the site involved in the disease pathology, or to which there is some other evidence of viral tropism. Peripheral blood mononuclear cells of an infected individual may also act as a carrier of an RFHV/KSHV subfamily virus. KSHV has been detected in PBMC of both Kaposi's Sarcoma (Moore et al. 1995b) and Castleman's disease (Dupin et al.). Other suitable sources are cell cultures developed from such sources, and enriched or isolated preparations of virus obtained from such sources. For negative control samples, tissue may be obtained from apparently unaffected sites on the same individuals, or from matched individuals who apparently do not suffer from the condition.

The process of identification of members of the RFHV/KSHV subfamily preferably involves the use of the methods and reagents provided in this invention, either singularly or in combination.

One method involves amplifying a polynucleotide encoding a herpes virus Glycoprotein B from DNA extracted from the sample. This can be performed, for example, by amplifying the polynucleotide in a reaction such as a PCR. In one variation, the amplification reaction is primed using broadly specific consensus-degenerate Type 1 oligonucleotides, such as those shown in Table 4. This will amplify herpes viruses, primarily of the gamma type. Since the RFHV/KSHV subfamily is a subset of gamma herpes viruses, Glycoprotein B sequences detected by this variation need to be characterized further to determine whether they fall into the RFHV/KSHV subfamily. In a second variation, the amplification is primed with RFHV or KSHV specific Type 3 oligonucleotides, such as those listed in Table 7, or other Glycoprotein B polynucleotide segments taken from these viruses. The amplification is conducted under conditions of medium to low stringency, so that the oligonucleotides will cross-hybridize with related species of viruses. In a more preferred variation, the amplification reaction is primed using RFHV/KSHV subfamily specific Type 2 oligonucleotides, such as those listed in Table 6. Under appropriate hybridization conditions, these primers will preferentially amplify Glycoprotein B from herpes viruses in the subfamily.

Preferred members of the subfamily detected using a Glycoprotein B polynucleotide probe are those that are at least 65% identical with the RFHV or KSHV Glycoprotein B nucleotide sequence between residues 36 and 354 of SEQ. ID NO:1 or SEQ. ID NO:3. More preferred are those that are at least about 67% identical; more preferred are those at least about 70% identical; more preferred are those that are at least about 80% identical; even more preferred are those about 90% identical or more.

Members of the subfamily can also be identified by performing a hybridization assay on the polynucleotide of the sample, using a suitable probe. The polynucleotide to be

tested may optionally be amplified before conducting the hybridization assay, such as by using Type 1 or Type 2 oligonucleotides as primers. The target is then tested in a hybridization reaction with a suitable labeled probe. The probe preferably comprises at least 21 nucleotides, preferably at least about 25 nucleotides, more preferably at least about 50 nucleotides contained the RFHV or KSHV Glycoprotein B sequence in SEQ. ID NOS:1 and 3. Even more preferably, the probe comprises nucleotides 36–354 of SEQ. ID NOS: 1 or 3. Other preferred probes include Type 2 oligonucleotides, such as those shown in Table 6. Hybridization conditions are selected to permit the probe to hybridize with Glycoprotein B polynucleotide sequences from the RFHV/KSHV subfamily, but not previously sequenced herpes viruses; particularly sHV1, bHV4, eHV2, mHV68, hEBV, hCMV, hHV6, hVZV, and HSV1. Formation of a stable duplex with the test polynucleotide under these conditions suggests the presence of a polynucleotide in the sample derived from a member of the RFHV/KSHV subfamily.

Members of the subfamily can also be identified by using a Class II antibody, the preparation of which was outlined earlier. A Class II antibody cross-reacts between antigens produced by members of the RFHV/KSHV subfamily, but not with other antigens, including those produced by herpes viruses not members of the subfamily. The test for new subfamily members is performed, for example, by using the antibodies in an immunohistochemistry study of tissue sections prepared from individuals with the conditions listed above. Positive staining of a tissue section with the antibody suggests the presence of Glycoprotein B in the sample from a member of the RFHV/KSHV subfamily, probably because the tissue is infected with the virus. If, in addition, the tissue section is non-reactive with RFHV and KSHV specific Class III antibodies, the Glycoprotein B in the tissue may be derived from another member of the subfamily. Similarly, if Class II antibodies are found in the circulation of an individual, the individual may have been subject to a present or past infection with a member of the RFHV/KSHV subfamily.

Once a putative new virus is identified by any of the aforementioned methods, its membership in the RFHV/KSHV subfamily may be confirmed by obtaining and sequencing a region of the Glycoprotein B gene of the virus, and comparing it with that of RFHV or KSHV according to the subfamily definition. For new members of the RFHV/KSHV subfamily, other embodiments of this invention may be brought into play for purposes of detection, diagnosis, and pharmaceutical development. Adaptation of the embodiments of the invention for a new subfamily member, if required, is expected to be minor in nature, and will be obvious based on the new sequence data, or a matter of routine adjustment.

Altered forms of Glycoprotein B from the RFHV/KSHV subfamily

This invention also embodies altered forms of Glycoprotein B of the RFHV/KSHV subfamily.

A number of studies on both naturally occurring and induced mutations of the Glycoprotein B of HSV1 and hCMV point to a role of certain regions of the molecule for its various biochemical functions. See, for example, Reschke et al. and Baghian et al. for a role of carboxy-terminal amino acids in fusion; Shiu et al. and Pellett et al. for epitopes for neutralizing antibodies; Gage et al. for regions of the molecule involved in syncytium formation; Navarro et al. (1992) for regions involved in virus penetration and cell-to-cell spread; Quadri et al. and Navarro et al.

(1991) for regions involved in intracellular transport of Glycoprotein B during biosynthesis.

Some of the residues described may be conserved between the Glycoprotein B molecules of the viruses investigated previously, and the Glycoprotein B molecules described herein. By analogy, mutation of the same residue in the Glycoprotein B of the RFHV/KSHV subfamily is expected to have a similar effect as described for other viruses. Alternatively, functional regions of different Glycoprotein B molecules may be combined to produce Glycoprotein B recombinants with altered function. For example, replacing the Glycoprotein B gene in a pathogenic virus with that of a non-pathogenic virus may reduce the pathogenicity of the recombinant (Kostal et al.). Either mutation and recombination of Glycoprotein B of the RFHV/KSHV herpes virus subfamily may lead to attenuated strains, in which either the infectivity, replication activity, or pathogenicity is reduced. Alterations in the Glycoprotein B sequence which have these effects are contemplated in this invention.

Attenuated strains of herpes viruses are useful, for example, in developing polyvalent vaccines. It is desirable, especially in developing countries, to provide prophylactic vaccines capable of stimulating the immune system against several potential pathogens simultaneously. Viruses that are engineered to express immunogenic peptides of several different pathogens may accomplish this purpose. Herpes viruses may be especially suitable vectors, because the large genome may easily accommodate several kilobases of extra DNA encoding the peptides. Ideally, the viral vector is sufficiently intact to exhibit some biological activity and attract the attention of the host's immune system, while at the same time being sufficiently attenuated not to cause significant pathology. Thus, an attenuated virus of the RFHV/KSHV subfamily may be useful as a vaccine against like virulent forms, and may be modified to express additional peptides and extend the range of immune protection.

Another use for attenuated forms of herpes viruses is as delivery vehicles for gene therapy (Latchman et al., Glorioso et al.). In order to be effective, polynucleotides in gene therapy must be delivered to the target tissue site. In the treatment of fibrotic diseases, malignancies and related conditions, attenuated viral vectors of the RFHV/KSHV subfamily may be preferable over other targeting mechanisms, including other herpes viruses, since they have the means by which to target towards the affected tissues. In this embodiment, the virus is first attenuated, and then modified to contain the polynucleotide that is desired for gene therapy, such as those that are outlined in a previous section.

Glycoprotein B in RFHV/KSHV subfamily vaccines

Because of its prominence on the envelope of the infectious virus and infected cells, glycoprotein B is predicted to be a useful target for immune effectors. Herpes virus Glycoprotein B is generally immunogenic, giving rise to antibodies capable of neutralizing the virus and preventing it from entering a replicative phase. In addition, Glycoprotein B is capable of eliciting a T-cell response, which may help eradicate an ongoing viral infection by attacking sites of viral replication in host cells.

This invention embodies vaccine compositions and methods for using them in the prevention and management of infection by viruses from the RFHV/KSHV subfamily.

One series of embodiments relate to active vaccines. These compositions are designed to stimulate an immune response in the individual being treated against Glycoprotein B. They generally comprise either the Glycoprotein B

molecule, an immunogenic fragment or variant thereof, or a cell or particle capable of expressing the Glycoprotein B molecule. Alternatively, they may comprise a polynucleotide encoding an immunogenic Glycoprotein B fragment (Horn et al.), preferably in the form of an expression vector. Polynucleotide vaccines may optionally comprise a delivery vehicle like a liposome or viral vector particle, or may be administered as naked DNA.

Vaccine compositions of this invention are designed in such a way that the immunogenic fragment is presented to stimulate the proliferation and/or biological function of the appropriate immune cell type. Compositions directed at eliciting an antibody response comprise or encode B cell epitopes, and may also comprise or encode other elements that enhance uptake and display by antigen-presentation cells, or that recruit T cell help. Compositions directed at eliciting helper T cells, especially CD4⁺ cells, generally comprise T cell epitopes that can be presented in the context of class II histocompatibility molecules. Compositions directed at stimulating cytotoxic T cells and their precursors, especially CD8⁺ cells, generally comprise T cell epitopes that can be presented in the context of class I histocompatibility molecules.

In the protection of an individual against a future exposure with herpes virus, an antibody response may be sufficient. Prophylactic compositions preferably comprise components that elicit a B cell response. Successful eradication of an ongoing herpes virus infection may involve the participation of cytotoxic T cells, T helper-inducer cells, or both. Infections for treating ongoing infection preferably comprise components capable of eliciting both T helper cells and cytotoxic T cells. Compositions that preferentially stimulate Type 1 helper (T_{H1}) cells over Type 2 helper (T_{H2}) cells are even more preferred. The preparation and testing of suitable compositions for active vaccines is outlined in the sections that follow.

Another series of embodiments relates to passive vaccines and other materials for adoptive transfer. These compositions generally comprise specific immune components against Glycoprotein B that are immediately ready to participate in viral neutralization or eradication. Therapeutic methods using these compositions are preferred to prevent pathologic consequences of a recent viral exposure. They are also preferred in immunocompromized individuals incapable of mounting a sufficient immune response to an active vaccine. Such individuals include those with congenital immunodeficiencies, acquired immunodeficiencies (such as those infected with HIV or on kidney dialysis), and those on immunosuppressive therapies, for example, with corticosteroids.

Suitable materials for adoptive transfer include specific antibody against Glycoprotein B, as described below. Also included are the adoptive transfer of immune cells. For example, T cells reactive against Glycoprotein B may be taken from a donor individual, optionally cloned or cultured in vitro, and then transferred to a histocompatible recipient. More preferably, the transferred cells are autologous to the recipient, and stimulated in vitro. Thus, T cells are purified from the individual to be treated, cultured in the presence of immunogenic components of Glycoprotein B and suitable stimulatory factors to elicit virus-specific cells, and then readministered.

Certain compositions embodied herein may have properties of both active and passive vaccines. For example, Glycoprotein B antibody given by adoptive transfer may confer immediate protection against herpes virus, and may also stimulate an ongoing response, through an anti-idiotypic network, or by enhancing the immune presentation of viral antigen.

Vaccines comprising Glycoprotein B polypeptides

Specific components of vaccines to stimulate an immune response against Glycoprotein B include the intact Glycoprotein B molecule, and fragments of Glycoprotein B that are immunogenic in the host.

Intact Glycoprotein B and longer fragments thereof may be prepared by any of the methods described earlier, especially purification from a suitable expression vector comprising a Glycoprotein B encoding polynucleotide. Isolated Glycoprotein B from other viral strains stimulate a protective immune response (See U.S. Pat. No. 5,171,568: Burke et al.). Preferred fragments comprise regions of the molecule exposed on the outside of the intact viral envelope; located within about 650 amino acids of the N-terminal of the mature protein.

Glycosylation of Glycoprotein B is not required for immunogenicity (O'Donnell et al.). Hence, glycosylated and unglycosylated forms of the molecule are equally preferred. Glycosylation may be determined by standard techniques; for example, comparing the mobility of the protein in SDS polyacrylamide gel electrophoresis before and after treating with commercially available endoglycosidase type F or H.

Smaller fragments of 5–50 amino acids comprising particular epitopes of Glycoprotein B are also suitable vaccine components. These may be prepared by any of the methods described earlier; most conveniently, by chemical synthesis. Preferred fragments are those which are immunogenic and expressed on the outside of the viral envelope. Even more preferred are fragments implicated in a biological function of Glycoprotein B, such as binding to cell surface receptors or penetration of the virus into a target cell.

Immunogenicity of various epitopes may be predicted by algorithms known in the art. Antigenic regions for B cell receptors may be determined, for example, by identifying regions of variable polarity (Hopp et al., see Example 9). Antigenic regions for T cell receptors may be determined, for example, by identifying regions capable of forming an amphipathic helix in the presentation groove of a histocompatibility molecule. Antigenic regions may also be identified by analogy with Glycoprotein B molecules of other viral species. See, e.g., Sanchez-Pescador et al. and Mester et al., for B cell epitopes of HSV1; Liu et al. for HLA-restricted helper T cell epitopes of hCMV; and Hanke et al. for cytotoxic T lymphocyte epitopes of HSV1.

Immunogenicity of various epitopes may be measured experimentally by a number of different techniques. Generally, these involve preparing protein fragments of 5–20 amino acids in length comprising potential antigenic regions, and testing them in a specific bioassay. Fragments may be prepared by CNBr and/or proteolytic degradation of a larger segment of Glycoprotein B, and purified, for example, by gel electrophoresis and blotting onto nitrocellulose (Demotz et al.). Fragments may also be prepared by standard peptide synthesis (Schumacher et al., Liu et al.). In a preferred method, consecutive peptides of 12 amino acids overlapping by 8 residues are synthesized according to the entire extracellular domain of the mature Glycoprotein B molecule, using F-Moc chemistry on a nylon membrane support (see Example 11).

Reactivity against the prepared fragment can then be determined in samples from individuals exposed to the intact virus or a Glycoprotein B component. The individual may have been experimentally exposed to the Glycoprotein B component by deliberate administration. Alternatively, the individual may have a naturally occurring viral infection, preferably confirmed by a positive amplification reaction

using a virus-specific oligonucleotide probe to Glycoprotein B or DNA Polymerase. Blood samples are obtained from the individual, and used to prepare serum, T cells, and peripheral blood mononuclear cells (PBMC) by standard techniques.

Serum may be tested for the presence of Glycoprotein B specific antibody in an enzyme-linked immunosorbant assay. For example, peptides attached to a solid support such as a nylon membrane are incubated with the serum, washed, incubated with an enzyme-linked anti-immunoglobulin, and developed using an enzyme substrate. The presence of antibody against a particular Glycoprotein B peptide is indicated by a higher level of reaction product in the test well than in a well containing an unrelated peptide (Example 11).

Lymphocyte preparations may be tested for the presence of Glycoprotein B specific helper T cells in a proliferation assay. Approximately 2×10^4 helper T cells are incubated with the peptide at 10^{-4} – 10^{-6} M in the presence of irradiated autologous or irradiated 10^5 PBMC as antigen presenting cells for about 3 days. [^3H]Thymidine is added for about the last 16 h of culture. The cells are then harvested and washed. Radioactivity in the washed cells at a level of about 10 fold over those cultured in the absence of peptide reflects proliferation of T cells specific for the peptide (Liu et al.). If desired, cells with a $\text{CD}3^+4^+8^-$ phenotype may be cloned for further characterization of the helper T cell response.

Lymphocyte preparations may be tested for the presence of Glycoprotein B specific cytotoxic T cells in a ^{51}Cr release assay. Targets are prepared by infecting allogeneic cells with a herpes virus comprising an expressible Glycoprotein B gene. Alternatively, allogeneic cells transfected with a Glycoprotein B expression vector may be used. The targets are incubated with ^{51}Cr for about 90 min at 37°C . and then washed. About 5×10^4 target cells are incubated with 10^{-4} – 10^{-5} M of the peptide and 0.1 – 2×10^4 test T cells for about 30 min at 37°C . Radioactivity released into the supernatant at a level substantially above that due to spontaneous lysis reflects CTL activity. If desired, cells with a $\text{CD}3^+4^+8^+$ phenotype may be cloned for further characterization of the CTL response.

Glycoprotein B peptides may optionally be combined in a vaccine with other peptides of the same virus. Suitable peptides include peptides of any of the other components of the herpes virus, such as Glycoproteins C, D, H, E, I, J, and G. Glycoprotein B peptides may also optionally be combined with immunogenic peptides from different viruses to provide a multivalent vaccine against more than one pathogenic organism. Peptides may be combined by preparing a mixture of the peptides in solution, or by synthesizing a fusion protein in which the various peptide components are linked.

Forms of Glycoprotein B comprising suitable epitopes may optionally be treated chemically to enhance their immunogenicity, especially if they comprise 100 amino acids or less. Such treatment may include cross-linking, for example, with glutaraldehyde; linking to a protein carrier, such as keyhole limpet hemocyanin (KLH) or tetanus toxoid.

The peptide or peptide mixture may be used neat, but normally will be combined with a physiologically and pharmacologically acceptable excipient, such as water, saline, physiologically buffered saline, or sugar solution.

In a preferred embodiment, an active vaccine also comprises an adjuvant which enhances presentation of the immunogen or otherwise accentuates the immune response against the immunogen. Suitable adjuvants include alum, aluminum hydroxide, beta-2 microglobulin (WO 91/16924:

Rock et al.), muramyl dipeptides, muramyl tripeptides (U.S. Pat. No. 5,171,568: Burke et al.), and monophosphoryl lipid A (U.S. Pat. No. 4,436,728: Ribic et al.; and WO 92/16231: Francotte et al.). Immunomodulators such as Interleukin 2 may also be present. The peptide and other components (if present) are optionally encapsulated in a liposome or microsphere. For an outline of the experimental testing of various adjuvants, see U.S. Pat. No. 5,171,568 (Burke et al.). A variety of adjuvants may be efficacious. The choice of an adjuvant will depend at least in part on the stability of the vaccine in the presence of the adjuvant, the route of administration, and the regulatory acceptability of the adjuvant, particularly when intended for human use.

Polypeptide vaccines generally have a broad range of effective latitude. The usual route of administration is intramuscular, but preparations may also be developed which are effective given by other routes, including intravenous, intraperitoneal, oral, intranasal, and by inhalation. The total amount of Glycoprotein B polypeptide per dose of vaccine when given intramuscularly will generally be about $10\text{ }\mu\text{g}$ to 5 mg; usually about $50\text{ }\mu\text{g}$ to 2 mg; and more usually about 100 to 500 μg . The vaccine is preferably administered first as a priming dose, and then again as a boosting dose, usually at least four weeks later. Further boosting doses may be given to enhance or rejuvenate the response on a periodic basis.

Vaccines comprising viral particles expressing Glycoprotein B

Active vaccines may also be prepared as particles that express an immunogenic epitope of Glycoprotein B.

One such vaccine comprises the L-particle of a recombinant herpes virus (see U.S. Pat. No. 5,284,122: Cunningham et al.). The genome of the recombinant virus is defective in a capsid component, or otherwise prevented from forming intact virus; however, it retains the ability to make L-particles. The genome is engineered to include a Glycoprotein B encoding polynucleotide of the present invention operatively linked to the controlling elements of the recombinant virus. The virus is then grown, for example, in cultured cells, and the particles are purified by centrifugation on a suitable gradient, such as FICOLLTM. Such preparations are free of infective virus, and capable of expressing peptide components of a number of different desirable epitopes.

Another such vaccine comprises a live virus that expresses Glycoprotein B of the present invention as a heterologous antigen. Such viruses include HIV, SIV, FIV, equine infectious anemia, visna virus, and herpes viruses of other species. The virus should be naturally non-pathogenic in the species to be treated; or alternatively, it should be attenuated by genetic modification, for example, to reduce replication or virulence. Herpes virus may be attenuated by mutation of a gene involved in replication, such as the DNA Polymerase gene. Herpes virus may also be attenuated by deletion of an essential late-stage component, such as Glycoprotein H (WO 92/05263: Inglis et al.). A live vaccine may be capable of a low level of replication in the host, particularly if this enhances protein expression, but not to the extent that it causes any pathological manifestation in the subject being treated.

A preferred viral species for preparing a live vaccine is adenovirus. For human therapy, human adenovirus types 4 and 7 have been shown to have no adverse affects, and are suitable for use as vectors. Accordingly, a Glycoprotein B polynucleotide of the present invention may be engineered, for example, into the E1 or E3 region of the viral genome. It is known that adenovirus vectors expressing Glycoprotein

B from HSV1 or HSV2 stimulate the production of high titer virus-neutralizing antibody (McDermott et al.). The response protects experimental animals against a lethal challenge with the respective live virus.

Also preferred as a virus for a live recombinant vaccine is a recombinant pox virus, especially vaccinia. Even more preferred are strains of vaccinia virus which have been modified to inactivate a non-essential virulence factor, for example, by deletion or insertion of an open reading frame relating to the factor (U.S. Pat. No. 5,364,773: Paoletti et al.). To prepare the vaccine, a Glycoprotein B encoding polynucleotide of the present invention is genetically engineered into the viral genome and expressed under control of a vaccinia virus promoter. Recombinants of this type may be used directly for vaccination at about 10^7 – 10^8 plaque-forming units per dose. Single doses may be sufficient to stimulate an antibody response. Vaccinia virus recombinants comprising Glycoprotein B of HSV1 are effective in protecting mice against lethal HSV1 infection (Cantin et al.).

Another vaccine in this category is a self-assembling replication-defective hybrid virus. See, for example, WO 92/05263 (Inglis et al.). The particle may contain, for example, capsid and envelope glycoproteins, but not an intact viral genome. As embodied in this invention, one of the glycoproteins in the viral envelope is Glycoprotein B.

In a preferred embodiment, the particle is produced by a viral vector of a first species, having a sufficient segment of the genome of that species to replicate, along with encoding regions for a capsid and an envelope from a heterologous species (see U.S. Pat. No. 5,420,026: Payne). Genetic elements of the first species are selected such that infection of eukaryotic cells with the vector produces capsid and envelope glycoproteins that self-assemble into replication-defective particles. In a variant of this embodiment, polynucleotides encoding the capsid and envelope glycoproteins are provided in two separate vectors derived from the first viral species. The capsid encoding regions may be derived from a lentivirus, such as HIV, SIV, FIV, equine infectious anemia virus, or visna virus. The envelope encoding regions comprise a Glycoprotein B encoding polynucleotide of the present invention. Preferably, all envelope components are encoded by a herpes virus, particularly of the RFHV/KSHV subfamily. The defective viral particles are obtained by infecting a susceptible eukaryotic cell line such as BSC-40 with the vector(s) and harvesting the supernatant after about 18 hours. Viral particles may be further purified, if desired, by centrifugation through a sucrose cushion. Particles may also be treated with 0.8% formalin at 40° C. for 24 hours prior to administration as a vaccine.

Vaccines comprising a live attenuated virus or virus analog may be lyophilized for refrigeration. Diluents may optionally include tissue culture medium, sorbitol, gelatin, sodium bicarbonate, albumin, gelatin, saline solution, phosphate buffer, and sterile water. Other active components may optionally be added, such as attenuated strains of measles, mumps, and rubella, to produce a polyvalent vaccine. The suspension may be lyophilized, for example, by the gas injection technique. This is performed by placing vials of vaccine in a lyophilizing chamber precooled to about -45° C. with 10–18 Pa of dry sterile argon, raising the temperature about 5–25° C. per h to +30° C., conducting a second lyophilizing cycle with full vacuum, and then sealing the vials under argon in the usual fashion (see EP 0290197B1: McAleer et al.). For vaccines comprising live herpes virus, the final lyophilized preparation will preferably contain 2–8% moisture.

It is recognized that a number of alternative compositions for active vaccines, not limited to those described here in

detail, may be efficacious in eliciting specific B- and T-cell immunity. All such compositions are embodied in the spirit of the present invention, providing they include a RFHV/KSHV subfamily Glycoprotein B polynucleotide or polypeptide as an active ingredient.

Vaccines comprising Glycoprotein B antibodies

Antibody against Glycoprotein B of the RFHV/KSHV subfamily may be administered by adoptive transfer to immediately confer a level of humoral immunity in the treated subject. Passively administered anti-glycoprotein B experimentally protects against a lethal challenge with other herpes viruses, even in subjects with compromised T-cell immunity (Eis-Hubinger et al.).

The antibody molecule used should be specific for Glycoprotein B against which protection is desired. It should not cross-reactive with other antigens, particularly endogenous antigens of the subject to be treated. The antibody may be specific for the entire RFHV/KSHV subfamily (Class II antibodies), or for a particular virus species (Class III antibodies), depending on the objective of the treatment. Preferably, the antibody will have an overall affinity for a polyvalent antigen of at least about 10^8 M⁻¹; more preferably it will be at least about 10^{10} M⁻¹; more preferably it will be at least about 10^{12} M⁻¹; even more preferably, it will be 10^{13} M⁻¹ or more. Intact antibody molecules, recombinants, fusion proteins, or antibody fragments may be used; however, intact antibody molecules or recombinants able to express natural antibody effector functions are preferred. Relevant effector functions include but are not limited to virus aggregation; antibody-dependent cellular cytotoxicity; complement activation; and opsonization.

Antibody may be prepared according to the description provided in an earlier section. For systemic protection, the antibody is preferably monomeric, and preferably of the IgG class. For mucosal protection, the antibody may be polymeric, preferably of the IgA class. The antibody may be either monoclonal or polyclonal; typically, a cocktail of monoclonal antibodies is preferred. It is also preferred that the preparation be substantially pure of other biological components from the original antibody source. Other antibody molecules of desired reactivity, and carriers or stabilizers may be added after purification.

In some instances, it is desirable that the antibody resemble as closely as possible an antibody of the species which is to be treated. This is to prevent the administered antibody from becoming itself a target of the recipient's immune response. Antibodies of this type are especially desirable when the subject has an active immune system, or when the antibodies are to be administered in repeat doses.

Accordingly, this invention embodies anti-Glycoprotein B antibody which is human, or which has been humanized. Polyclonal human antibody may be purified from the sera of human individuals previously infected with the respective RFHV/KSHV subfamily herpes virus, or from volunteers administered with an active vaccine. Monoclonal human antibody may be produced from the lymphocytes of such individuals, obtained, for example, from peripheral blood. In general, human hybridomas may be generated according to the methods outlined earlier. Usually, the production of stable human hybridomas will require a combination of manipulative techniques, such as both fusion with a human myeloma cell line and transformation, for example, with EBV.

In a preferred method, human antibody is produced from a chimeric non-primate animal with functional human immunoglobulin loci (WO 91/10741: Jakobovits et al.). The non-primate animal strain (typically a mouse) is incapable of

expressing endogenous immunoglobulin heavy chain, and optimally at least one endogenous immunoglobulin light chain. The animals are genetically engineered to express human heavy chain, and optimally also a human light chain. These animals are immunized with a Glycoprotein B of the RFHV/KSHV subfamily of herpes viruses. Their sera can then be used to prepare polyclonal antibody, and their lymphocytes can be used to prepare hybridomas in the usual fashion. After appropriate selection and purification, the resultant antibody is a human antibody with the desired specificity.

In another preferred method, a monoclonal antibody with the desired specificity for Glycoprotein B is first developed in another species, such as a mouse, and then humanized. To humanize the antibody, the polynucleotide encoding the specific antibody is isolated, antigen binding regions are obtained, and then recombined with polynucleotides encoding elements of a human immunoglobulin of unrelated specificity. Alternatively, the nucleotide sequence of the specific antibody is obtained and used to design a related sequence with human characteristics, which can be prepared, for example, by chemical synthesis. The heavy chain constant region or the light chain constant region of the specific antibody, preferably both, are substituted with the constant regions of a human immunoglobulin of the desired class. Preferably, segments of the variable region of both chains outside the complementarity determining regions (CDR) are also substituted with their human equivalents (EP 0329400: Winter).

Even more preferably, segments of the variable region are substituted with their human equivalents, providing they are not involved either in antigen binding or maintaining the structure of the binding site. Important amino acids may be identified, for example, as described by Padlan. In one particular technique (WO 94/11509: Couto et al.), a positional consensus sequence is developed using sequence and crystallography data of known immunoglobulins. The amino acid sequence of the Glycoprotein B specific antibody is compared with the model sequence, and amino acids involved in antigen binding, contact with CDR's, or contact with opposing chains are identified. The other amino acids are altered, where necessary, to make them conform to a consensus of human immunoglobulin sequences. A polynucleotide encoding the humanized sequence is then prepared, transfected into a host cell, and used to produce humanized antibody with the same Glycoprotein B specificity as the originally obtained antibody clone.

Specific antibody obtained using any of these methods is generally sterilized, mixed with a pharmaceutically compatible excipient. Stabilizers such as 0.3 molar glycine, and preservatives such as 1:10,000 thimerosal, may also be present. The suspension may be buffered to neutral pH (~7.0), for example, by sodium carbonate. The potency may optionally be adjusted by the addition of normal human IgG, obtained from large pools of normal plasma, for example, by the Cohn cold ethanol fractionation procedure. Other diluents, such as albumin solution, may be used as an alternative. The concentration is adjusted so that a single dose administration constitutes 0.005–0.2 mg/kg, preferably about 0.06 mg/kg. A single dose preferably results in a circulating level of anti-Glycoprotein B, as detected by ELISA or other suitable technique, which are comparable to those observed in individuals who have received an active Glycoprotein B vaccine or have recovered from an acute infection with the corresponding virus, or which are known from experimental work to be protective against challenges with a pathologic dose of virus.

Administration should generally be performed by intramuscular injection, not intravenously, and care should be taken to assure that the needle is not in a blood vessel. Special care should be taken with individuals who have a history of systemic allergic reactions following administration of human globulin. For prophylactic applications, the antibody preparation may optionally be administered in combination with an active vaccine for Glycoprotein B, as described in the preceding sections. For post-exposure applications, the antibody preparation is preferably administered within one week of the exposure, more preferably within 24 hours, or as soon as possible after the exposure. Subsequent doses may optionally be given at approximately 3 month intervals.

As for all therapeutic instruments described herein, the amount of composition to be used, and the appropriate route and schedule of administration, will depend on the clinical status and requirements of the particular individual being treated. The choice of a particular regimen is ultimately the responsibility of the prescribing physician or veterinarian.

The foregoing description provides, inter alia, a detailed explanation of how Glycoprotein B encoding regions of herpes viruses, particularly those of the RFHV/KSHV subfamily, can be identified and their sequences obtained. Polynucleotide sequences for encoding regions of Glycoprotein B of both RFHV and KSHV are provided.

The polynucleotide sequences listed herein for RFHV and KSHV are believed to be an accurate rendition of the sequences contained in the polynucleotides from the herpes viruses in the tissue samples used for this study. They represent a consensus of sequence data obtained from multiple clones. However, it is recognized that sequences obtained by amplification methods such as PCR may comprise occasional errors in the sequence as a result of amplification. The error rate is estimated to be between about 0.44% and 0.75% for single determinations; about the same rate divided by $\sqrt{n-1}$ for the consensus of n different determinations. Nevertheless, the error rate may be as high as 1% or more. Sequences free of amplification errors can be obtained by creating a library of herpes virus polynucleotide sequences, using oligonucleotides such as those provided in Table 7 to select relevant clones, and sequencing the DNA in the selected clones. The relevant methodology is well known to a practitioner of ordinary skill in the art, who may also wish to refer to the description given in the Example section that follows.

It is recognized that allelic variants and escape mutants of herpes viruses occur. Polynucleotides and polypeptides may be isolated or derived that incorporate mutations, either naturally occurring, or accidentally or deliberately induced, without departing from the spirit of this invention.

The examples presented below are provided as a further guide to a practitioner of ordinary skill in the art, and are not meant to be limiting in any way.

EXAMPLES

Example 1

Oligonucleotide Primers for Herpes Virus Glycoprotein B

Amino acid sequences of known herpes virus Glycoprotein B molecules were obtained from the PIR protein database, or derived from DNA sequences obtained from the GenBank database. The sequences were aligned by computer-aided alignment programs and by hand.

Results are shown in FIG. 3. sHV1, bHV4, mHV68, EBV and hHV6 sequences were used to identify regions that were

relatively well conserved, particularly amongst the gamma herpes viruses. Nine regions were chosen for design of amplification primers. The DNA sequences for these regions were then used to design the oligonucleotide primers. The primers were designed to have a degenerate segment of 8–14 base pairs at the 3' end, and a consensus segment of 18–30 bases at the 5' end. This provides primers with optimal sensitivity and specificity.

The degenerate segment extended across highly conserved regions of herpes virus Glycoprotein B sequences, encompassing the least number of alternative codons. The primers could therefore be synthesized with alternative nucleotide residues at the degenerate positions and yield a minimum number of combinations. There were no more than 256 alternative forms for each of the primers derived.

The consensus segment was derived from the corresponding flanking region of the Glycoprotein B sequences. Generally, the consensus segment was derived by choosing the most frequently occurring nucleotide at each position of all the Glycoprotein B sequences analyzed. However, selection was biased in favor of C or G nucleotides, to maximize the ability of the primers to form stable duplexes.

Results are shown in FIGS. 4–12, and are summarized in Table 4. In a PCR, oligonucleotides listed in Table 4 as having a “sense” orientation would act as primers by hybridizing with the strand antisense to the coding strand, and initiating polymerization in the same direction as the Glycoprotein B encoding sequence. Oligonucleotides listed in Table 4 as having an “antisense” orientation would hybridize with the coding strand and initiate polymerization in the direction opposite to that of the Glycoprotein B encoding sequence.

Synthetic oligonucleotides according to the designed sequences were ordered and obtained from Oligos Etc, Inc.

Example 2

DNA Extraction

Biopsy specimens were obtained from Kaposi's sarcoma lesions from human subjects diagnosed with AIDS. The specimens were fixed in paraformaldehyde and embedded in paraffin, which were processed for normal histological examination.

Fragments of the paraffin samples were extracted with 500 μ L of xylene in a 1.5 mL EPPENDORF™ conical centrifuge tube. The samples were rocked gently for 5 min at room temperature, and the tubes were centrifuged in an EPPENDORF™ bench-top centrifuge at 14,000 rpm for 5 min. After removing the xylene with a Pasteur pipette, 500 μ L of 95% ethanol was added, the sample was resuspended, and then re-centrifuged. The ethanol was removed, and the wash step was repeated. Samples were then air-dried for about 1 hour. 500 μ L of proteinase-K buffer (0.5% TWEEN™ 20, a detergent; 50 mM Tris buffer pH 7.5, 50 mM NaCl) and 5 μ L of proteinase K (20 mg/mL) were added, and the sample was incubated for 3 h at 55° C. The proteinase K was inactivated by incubating at 95° C. for 10 min.

Samples of DNA from KS tissue were pooled to provide a consistent source of polynucleotide for the amplification reactions. This pool was known to contain DNA from KSHV, as detected by amplification of KSHV DNA polymerase sequences, as described in commonly owned U.S. patent application Ser. No. 60/001,148.

Example 3

Obtaining Amplified Segments of KSHV Glycoprotein B

The oligonucleotides obtained in Example 1 were used to amplify segments of the DNA extracted from KSHV tissue in Example 2, according to the following protocol.

A first PCR reaction was conducted using 2 μ L of pooled DNA template, 1 μ L of oligonucleotide FRFDA (50 pmol/ μ L), 1 μ L of oligonucleotide TVNCB (50 pmol/ μ L), 10 μ L of 10 \times buffer, 1 μ L containing 2.5 mM of each of the deoxyribonucleotide triphosphates (dNTPs), 65 μ L distilled water, and 65 μ L mineral oil. The mixture was heated to 80° C. in a Perkin-Elmer (model 480) PCR machine. 0.5 μ L Taq polymerase (BRL, 5 U/ μ L) and 19.5 μ L water was then added. 35 cycles of amplification were conducted in the following sequence: 1 min at 94° C., 1 min at the annealing temperature, and 1 min at 72° C. The annealing temperature was 60° C. in the first cycle, and decreased by 2° C. each cycle until 50° C. was reached. The remaining cycles were performed using 50° C. as the annealing temperature.

A second PCR reaction was conducted as follows: to 1 μ L of the reaction mixture from the previous step was added 1 μ L oligonucleotide NIVPA (50 pmol/ μ L), 1 μ L oligonucleotide TVNCB (50 pmol/ μ L), 10 μ L of 10 \times buffer, 1 μ L dNTPs, 66 μ L water, and 65 μ L mineral oil. The mixture was heated to 80° C., and 0.5 μ L Taq polymerase in 19.5 μ L water was added. 35 cycles of amplification were conducted using the same temperature step-down procedure as before. The PCR product was analyzed by electrophoresing on a 2% agarose gel and staining with ethidium bromide.

The two-round amplification procedure was performed using fourteen test buffers. Five buffers yielded PCR product of about the size predicted by analogy with other herpes sequences. These included WB4 buffer (10 \times WB4 buffer is 0.67 M Tris buffer pH 8.8, 40 mM MgCl₂, 0.16 M (NH₄)₂SO₄, 0.1 M β -mercaptoethanol, 1 mg/mL bovine serum albumin, which is diluted 1 to 10 in the reaction). Also tested was WB2 buffer (the same as WB4 buffer, except with 20 mM MgCl₂ in the 10 \times concentrate). Also tested were buffers that contained 10 mM Tris pH 8.3, 3.5 mM MgCl₂ and 25 mM KCl; or 10 mM Tris pH 8.3, 3.5 mM MgCl₂ and 75 mM KCl; or 10 mM Tris pH 8.8, 3.5 mM MgCl₂ and 75 mM KCl; when diluted to final reaction volume. The WB4 buffer showed the strongest band, and some additional fainter bands. This may have been due to a greater overall amount of labeled amplified polynucleotide in the WB4 sample.

The product from amplification with WB2 buffer was selected for further investigation. A third round of amplification was performed to introduce a radiolabel. The last-used oligonucleotide (TVNCB) is end-labeled with gamma ³²P-ATP, and 1 μ L was added to 20 μ L of the reaction mixture from the previous amplification step, along with 1 μ L 2.5 mM dNTP. The mixture was heated to 80° C., and 0.5 μ L Taq polymerase was added. Amplification was conducted through five cycles of 94° C., 60° C. and 72° C. The reaction was stopped using 8.8 μ L of loading buffer from a Circumventing sequencing gel kit.

A ~4 μ L aliquot of the radiolabeled reaction product was electrophoresed on a 6% polyacrylamide sequencing gel for 1.5 h at 51° C. The gel was dried for 1.5 h, and an autoradiograph was generated by exposure for 12 h. Two bands were identified. The larger band had the size expected for the fragment from analogy with other gamma herpes virus sequences.

The larger band was marked and cut out, and DNA was eluted by incubation in 40 μ L TE buffer (10 mM Tris and 1 mM EDTA, pH. 8.0). A further amplification reaction was performed on the extracted DNA, using 1 μ L of the eluate, 10 μ L 10 \times WB2 buffer, 1 μ L 2.5 mM dNTP, 1 μ L of each of the second set of oligonucleotide primers (NIVPA and TVNCB), and 65 μ L water. The mixture was heated to 80° C., and 0.5 μ L Taq polymerase in 19.5 μ L water was added.

Amplification was conducted through 35 cycles, using the temperature step-down procedure described earlier.

Example 4

Sequence of the 386 Base Fragment of KSHV Glycoprotein B

The amplified polynucleotide fragment from the Glycoprotein B gene of KSHV was purified and cloned according to the following procedure.

40 μ L of amplification product was run on a 2% agarose gel, and stained using 0.125 μ g/mL ethidium bromide. The single band at about 400 base pairs was cut out, and purified using a QIAGEN™ II gel extraction kit, according to manufacturer's instructions.

The purified PCR product was ligated into the pGEM™-t cloning vector. The vector was used to transform competent bacteria (*E. coli* JM-109). Bacterial clones containing the amplified DNA were picked and cultured. The bacteria were lysed and the DNA was extracted using phenol-chloroform followed by precipitation with ethanol. Colonies containing inserts of the correct size were used to obtain DNA for sequencing. The clone inserts were sequenced from both ends using vector-specific oligonucleotides (forward and reverse primers) with a SEQUENASE™ 7-deaza dGTP kit, according to manufacturer's directions. A consensus sequence for the new fragment was obtained by combining sequence data obtained from 5 clones of one KSHV Glycoprotein B amplification product.

The length of the fragment in between the primer hybridizing regions was 319 base pairs. The nucleotide sequence is listed as SEQ. ID NO:3 and shown in FIG. 1. The encoded polypeptide sequence is listed as SEQ. ID NO:4.

FIG. 13 compares the sequence of this Glycoprotein B gene fragment with the corresponding sequence of other gamma herpes viruses. Single dots (.) indicate residues in other gamma herpes viruses that are identical to those of the KSHV sequence. Dashes (-) indicate positions where gaps have been added to provide optimal alignment of the encoded protein. The longest stretch of consecutive nucleotides that is identical between the KSHV sequence and any of the other listed sequences is 14. Short conserved sequences are scattered throughout the fragment. Overall, the polynucleotide fragment is 63% identical between KSHV and the two closest herpes virus sequences, SHV1 and bHV4.

The sequence data was used to design Type 3 oligonucleotide primers of 2040 base pairs in length. The primers were designed to hybridize preferentially with the KSHV Glycoprotein B polynucleotide, but not with other sequenced polynucleotides encoding Glycoprotein B. Example primers of this type were listed earlier in Table 7.

FIG. 14 compares the predicted amino acid sequence encoded by the same Glycoprotein B gene fragment. At the amino acid level, two short segments are shared between KSHV and a previously known gamma herpes virus, bHV4. The first (SEQ. ID NO:64) is 13 amino acids in length and located near the N-terminal end of the fragment. The second (SEQ. ID NO:65) is 15 amino acids in length and located near the C-terminal end of the fragment. All other segments shared between KSHV and other gamma herpes viruses are 9 amino acids or shorter.

Example 5

Sequence of the 386 Base Fragment of RFHV Glycoprotein B

Tissue specimens were obtained from the tumor of a *Macaque nemestrina* monkey at the University of Washing-

ton Regional Primate Research Center. The specimens were fixed in paraformaldehyde and embedded in paraffin. DNA was extracted from the specimens according to the procedure of Example 2.

The presence of RFHV polynucleotide in DNA preparations was determined by conducting PCR amplification reactions using oligonucleotide primers hybridizing to the DNA polymerase gene. Details of this procedure are provided in commonly owned U.S. patent application Ser. No. 60/001,148. DNA extracts containing RFHV polynucleotide determined in this fashion were pooled for use in the present study.

DNA preparations containing RFHV polynucleotide served as the template in PCR amplification reactions using Glycoprotein B consensus-degenerate oligonucleotides FRFDA and TVNCB, followed by a second round of amplification using oligonucleotides NIVPA and TVNCB. Conditions were essentially the same as in Example 3, except that only WB4 buffer produced bands of substantial intensity, with the amount of DNA in the initial source and the conditions used. Labeling of the amplified DNA was performed with ³²P end-labeled NIVPA, as before; the product was electrophoresed on a 6% polyacrylamide gel, and an autoradiogram was obtained. A ladder of bands corresponding to about 386 base pairs and about 10 higher mol wt concatemers was observed. The 386 base pair band (with the same mobility as a simultaneously run KSHV fragment) was cut out of the gel and extracted.

To determine whether the DNA in this extract was obtained from a specific amplification reaction, PCR's were set up using NIVPASQ alone, TVNCBSQ alone, or the two primers together. Buffer conditions were the same as for the initial amplification reactions. The mixture was heated to 80° C., Taq polymerase was added, and the amplification was carried through 35 cycles using the temperature step-down procedure. Theoretically, specific amplification reactions accumulate product linearly when one primer is used, and exponentially when using two primers with opposite orientation. Thus, specificity is indicated by more product in the reaction using both primers, whereas equal product in all three mixtures suggests non-specific amplification. Amplification products from these test reactions were analyzed on an agarose gel stained with ethidium bromide. The RF extract showed no product for the NIVPASQ reaction, a moderate staining band for the TVNCBSQ reaction at the appropriate mobility, and an intensely staining band for both primers together. For a KSHV fragment assayed in parallel, there was a faint band for the NIVPASQ reaction, no band for the TVNCBSQ reaction, and an intensely staining band for both primers together. We concluded that the 386 base pair band in the RF extract represented specific amplification product.

Accordingly, 40 μ L of the RF extract that had been amplified with both primers was run preparatively on a 2% agarose gel, and the ~386 base pair band was cut out. Agarose was removed using a QIAGEN™ kit, and the product was cloned in *E. coli* and sequenced as in Example 4. A consensus sequence was determined for 3 different clones obtained from the same amplified RFHV product.

The polynucleotide sequence of RFHV Glycoprotein B fragment (SEQ. ID NO:1) is aligned in FIG. 1 with the corresponding sequence from KSHV. Also shown is the encoded RFHV amino acid sequence (SEQ. ID NO:2). Between the primer hybridization regions (nucleotides 36–354), the polynucleotide sequences are 76% identical; and the amino acid sequences are 91% identical. The inter-

nal cysteine residue and the potential N-linked glycosylation site are both conserved between the two viruses.

The sequence data was used to design Type 3 oligonucleotide primers of 20–40 base pairs in length. The primers were designed to hybridize preferentially with the RFHV Glycoprotein B polynucleotide, but not with other sequenced polynucleotides encoding Glycoprotein B. Example primers of this type were listed earlier in Table 7.

FIG. 15 compares the predicted amino acid sequence encoded by nucleotides 36–354 of the Glycoprotein B gene fragment. As for the KSHV sequence, two short segments are shared between RFHV and a previously known gamma herpes virus, bHV4. All other segments shared between RFHV and other gamma herpes viruses are shorter than 9 amino acids in length.

FIG. 16 is an alignment of sequence data for the same Glycoprotein B fragment in the spectrum of herpes viruses for which data is available. FIG. 17 shows the phylogenetic relationship between herpes viruses, based on the degree of identity across the partial Glycoprotein B amino acid sequences shown in FIG. 16. By amino acid homology, amongst the viruses shown, RFHV and KSHV are most closely related to bHV4, eHV2, and sHV1.

Example 6

Oligonucleotide Primers and Probes for the RFHV/KSHV Subfamily

Based on the polynucleotide fragment obtained for RFHV and KSHV, seven Type 2 oligonucleotides were designed that could be used either as PCR primers or as hybridization probes with members of the RFHV/KSHV subfamily.

Four consensus-degenerate Type 2 oligonucleotides, SHMDA, CFSSB, ENTEA, and DNIQB are shown in FIG. 17, alongside the sequences they were derived from. Like the oligonucleotides of Example 1, they have a consensus segment towards the 5' end, and a degenerate segment towards the 3' end. However, these oligonucleotides are based only on the RFHV and KSHV sequences, and will therefore preferentially form stable duplexes with Glycoprotein B of the RFHV/KSHV subfamily. A list of exemplary Type 2 oligonucleotides was provided earlier in Table 6.

Different Type 2 oligonucleotides have sense or antisense orientations. Primers with opposing orientations may be used together in PCR amplifications. Alternatively, any Type 2 oligonucleotide may be used in combination with a Type 1 oligonucleotide with an opposite orientation.

Example 7

Upstream and Downstream Glycoprotein B Sequence

Further amplification reactions are conducted to obtain additional sequence data. The source for KSHV DNA is Kaposi's Sarcoma tissue, either frozen tissue blocks or paraffin-embedded tissue, prepared according to Example 2, or cell lines developed from a cancer with a KSHV etiology, such as body cavity lymphoma. Also suitable is KSHV that is propagated in culture (Weiss et al.)

The general strategy to obtain further sequence data in the 5' direction of the coding strand is to conduct amplification reactions using the consensus-degenerate (Type 1) oligonucleotide hybridizing upstream from the fragment as the 5' primer, in combination with the closest virus-specific (Type

3) oligonucleotides as the 3' primers. Thus, a first series of amplification cycles are conducted, for example, using FRFDA and TNKYB as the first set of primers. This may optionally be followed by a second series of amplification cycles, conducted, for example, using FRFDA and GLTEB as a second set of primers.

The conditions used are similar to those described in Examples 3 and 4. The reaction is performed in WB4 buffer, using the temperature step-down procedure described in Example 3. After two rounds of amplification, the product is labeled using the last-used virus-specific oligonucleotide (GLTEB, in this case), end-labeled with gamma ³²P-ATP. The labeled product is electrophoresed on 6% polyacrylamide, and a band corresponding to the appropriate size as predicted by analogy with other herpes viruses is excised. After re-amplification, the product is purified, cloned, and sequenced as before. A consensus sequence for the new fragment is obtained by combining results of about three determinations.

In order to obtain further sequence data in the 3' direction of the coding strand, amplifications are conducted using consensus-degenerate (Type 1) oligonucleotides hybridizing downstream from the fragment as the 3' primer, in combination with the closest virus-specific (Type 3) oligonucleotides as the 5' primers. In one example, a first series of amplification cycles are conducted using NVFDB and TVFLA, optionally followed by a second series conducted using NVFDB and SQPVA. Amplification and sequencing is performed as before. The new sequence is used to design further Type 3 oligonucleotides with a sense orientation, which are used with other downstream-hybridizing Type 1 oligonucleotides (such as FREYB and NVFDB) to obtain further sequence data. Alternatively, further sequence data in the 3' direction is obtained using Type 1 oligonucleotides with opposite orientation: for example, two primers are selected from the group of FRFDA, NIVPA, TVNCA, NIDFB, NVFDB, and FREYB; additional primers may be selected for nested amplification.

To obtain sequence data 3' from the most downstream oligonucleotide primer, Type 1 primers such as CYSRA, or Type 3 primers such as TVFLA, may be used in combination with primers hybridizing towards the 5' end of the DNA polymerase gene. Oligonucleotide primers hybridizing to the DNA polymerase gene of herpes viruses related to RFHV and KSHV are described in commonly owned U.S. patent application Ser. No. 60/001,148. The DNA polymerase encoding region is located 3' to the Glycoprotein B encoding region. PCRs conducted using this primer combination are expected to amplify polynucleotides comprising the 3' end of the Glycoprotein B encoding region, any intervening sequence if present, and the 5' end of the DNA Polymerase encoding region.

This strategy was implemented as follows:

DNA containing KSHV encoding sequences for Glycoprotein B was prepared from a frozen Kaposi's sarcoma sample, designated RiGr, and a cell line derived from a body cavity lymphoma, designated BC-1.

In order to obtain the full 5' sequence, a Type 1 oligonucleotide probe was designed for the encoding sequence suspected of being upstream of Glycoprotein B: namely, the capsid maturation gene (CAPMAT). Known sequences of CAPMAT from other viruses were used to identify a relatively conserved region, and design a consensus-degenerate primer designated FENSA to hybridize with CAPMAT in the sense orientation of Glycoprotein B. A Type 1 oligonucleotide probe was designed for the encoding sequence

suspected of being downstream of Glycoprotein B: namely, the DNA polymerase. These oligonucleotides are listed in Table 9:

TABLE 9

Additional Type 1 Oligonucleotides used for Detecting, Amplifying, or Characterizing Herpes Virus Polynucleotides					
Designation	Sequence (5' to 3')	Length	No. of forms	Orientation	SEQ ID:
Target: Capsid/Maturation gene from Herpes Viruses, especially from gamma Herpes Viruses					
FENSAC	GCCTTTGAGAATTCYAARTAYATHAAR	27	48	sense	77
FENSAG	GGGUTGAGAAUCYAARTAYATHAAR	27	48	sense	78
Target: DNA polymerase gene from Herpes Viruses, especially from gamma Herpes Viruses					
CVNVB	TAAAAGTACAGCTCCTGCCGAANACRTTNAC RCA	35	64	antisense	79

Amplification was carried out using pairs of sense and antisense primers that covered the entire Glycoprotein B encoding region. Fragments obtained include those listed in Table 10.

TABLE 10

KSHV Glycoprotein B fragments obtained			
Fragment	Length	Position	
1 NIVPA → TVNCB	0.39 kb	original fragment	
2 FENSA → VNVNB	0.9 kb	5' of fragment 1 across to CAPMAT	
3 TVNCA → FREYB	2.3 kb	3' of fragment 1'	
4 FAYDA → FREYB	0.65 kb	3' of fragment 1	
5 SQPVA → HVLQB	2.5 kb	3' of fragment 1 across to DNA polymerase	
6 FREYA → SCGFB	1.1 kb	3' of fragment 2 across to DNA polymerase	

The protocol used for amplifying and sequencing was as follows: PCR amplification was carried out using the DNA template with the primer pair (e.g., FREYA and SCGFB). 35 cycles were conducted of 94° C. for 45 sec. 60° C. for 45 sec. and 72° C. for 45 sec; and then followed by a final extension step at 72° C. for 10 min. PCR products of the predicted

length were purified on agarose gels using the QIAQUICK™ PCR purification kit from Qiagen. Purified PCR products were reamplified in a second round of ampli-

fication. The second round was conducted alternatively in a nested or non-nested fashion. In the example given, second-round amplification was conducted using FREYA and SCGFB, or with FREYA and HVLQB. Amplification for 35 cycles was conducted at 94° C. for 45 sec, 65° C. for 45 sec, and 72° C. for 45 sec; and then followed by a final extension step at 72° C. for 60 min.

The PCR products were ligated into the Novagen PT7 BLUE™ vector, and transformed into Novablue competent *E. coli*. Ligations and transformations were performed using Novagen protocols. Colonies were screened by PCR using M13 forward and reverse oligonucleotides. Using the Quiaquick plasmid isolation kit, plasmids were isolated from PCR positive colonies that had been grown up overnight in 5 mL LB broth at 37° C. Manual sequencing of the plasmids using M13 forward and reverse sequencing primers was performed following the USB Sequenase Kit protocol (USB). Automated sequencing was performed by ABI methods.

Additional KSHV-specific Type 3 oligonucleotides were designed as the KSHV sequence emerged. Type 3 oligonucleotides were used in various pair combinations or with Type 1 oligonucleotides to PCR amplify, clone, and sequence sections of the KSHV DNA. The Type 3 oligonucleotides used are listed in Table 11:

TABLE 11

Additional Type 3 Oligonucleotides used for Detecting, Amplifying, or Characterizing Herpes Virus Polynucleotides encoding Glycoprotein B					
Designation	Sequence (5' to 3')	Length	No. of forms	Orientation	SEQ ID:
Target: Glycoprotein B from KSHV					
GAYTA	TGTGGAACGGGAGCGTACAC	21	1	sense	80
DTYSB	TCAGACAAGAGTACGTGTCGG	21	1	anti-sense	81
AIYGB	TACAGGTCGACCGTAGATGGC	21	1	ariti-sense	82
VTECA	CGCCATTTCCGTGACCGAGTG	21	1	sense	83
CEHYB	TGATGAAGTAGTGTTCCGAGG	21	1	anti-sense	84
DLGGB	GATGCCACCCAGGTCCGCCAC	21	1	anti-sense	85
DLGGA	GTGGCGGACCTGGGTGGCATC	21	1	sense	86
RAPPA	CGTAGATCGCAGGGCACCTCC	21	1	sense	87
Target: DNA Polymerase from KSHV					
GEVFB	GTCTCTCCCGCAATACTTCT	21	1	antisense	88
HVLQB	GAGGGCTGCTGGAGGACGTG	21	1	antisense	89
SCGFB	CGGTGGAGAAGCCGAGGATG	21	1	antisense	90

FIG. 18 is a map showing the location where oligonucleotides hybridize with the KSHV DNA. Abbreviations used are as follows: d or h=consensus-degenerate probes that hybridize with herpesvirus sequences (Type 1), sq=additional sequencing tail available, g=probes that hybridize with gamma herpesviruses (Type 1), f=probes that hybridize with KSHV/RFHV family of herpesviruses (Type 2), ks=probes specific for KSHV (Type 3).

FIG. 19 lists a consensus sequence obtained by compiling sequence data from each of the characterized fragments. The polynucleotide sequence (SEQ. ID NO:91) is shown. Nucleotides 1–3056 (SEQ. ID NO:92) incorporating the region before the DNA polymerase encoding sequence is an embodiment of this invention. This consensus sequence represents the consensus of data obtained from both the Kaposi's sarcoma sample RiGr, and the lymphoma cell line BC-1, with a plurality of clones being sequenced for each sample and each gene segment. Between about 3–9 determinations have been performed at each location.

Also shown in FIG. 19 is the amino acid translation of the three open reading frames (SEQ. ID NOS:93–95). The encoded CAPMAT protein fragment (SEQ. ID NO:93) overlaps the 5' end of the Glycoprotein B encoding sequence (SEQ. ID NO:94) in a different phase. Further upstream, the CAPMAT encoding sequence is also suspected of comprising control elements for Glycoprotein B transcription, due to homology with the binding site for RNA polymerase 2 of Epstein Barr Virus. This putative promoter region is underlined in the Figure. At the 3' end of the Glycoprotein B encoding sequence, there is an untranslated sequence including a polyadenylation signal. Further downstream is the encoding sequence for a DNA Polymerase fragment (SEQ. ID NO:95).

When the Glycoprotein B encoding sequence was compared with other sequences on GenBank, homology was found only with Glycoprotein B sequences from other herpes viruses. Occasional sequences of 20 nucleotides or less are shared with several herpes viruses. The sequence ATGTTTCAGGGAGTACAACACTACTACAC (SEQ. ID NO:98) is shared with eHV2. Other than this sequence, segments of the KSHV encoding region 21 nucleotides or longer are apparently unique, compared with other previously known sequences.

Within the Glycoprotein B encoding sequence, four allelic variants were noted at the polynucleotide level between sequence data obtained using the Kaposi's sarcoma sample and that obtained using the body cavity lymphoma cell line. These are indicated in the Figure by arrows. All but one of the variants was silent. The fourth variant causes a difference of Proline to Leucine in the gene product.

The protein product encoded by the KSHV Glycoprotein B gene has the following features: There is a domain at the N-terminus that corresponds to the signal-peptide domain (the "leader") of Glycoprotein B other herpes viruses. The complete KSHV Glycoprotein B amino acid sequence with that known for other herpes viruses is provided in FIG. 3, and reveals areas of homology. Residues highly conserved amongst herpes virus Glycoprotein B sequences are marked with an asterisk (*). The cysteine residues conserved amongst other herpes virus Glycoprotein B sequences are also present in that of KSHV. In addition, there are two additional cysteines which could form an additional internal disulfide and stabilize the three-dimensional structure (marked by "●"). The KSHV Glycoprotein B sequence also has a predicted membrane-spanning domain that corresponds to that on Glycoprotein B of other herpes viruses.

Another feature of the KSHV Glycoprotein B is the presence of an RGD triplet near the N-terminal of the mature protein. The same triplet is present in proteins such as fibrinogen, fibronectin, vitronectin, thrombospondin, osteopontin, and laminin, and has been shown to direct binding of these proteins to cell surfaces via integrin receptors. The RGD domain of laminin has been shown to bind to endothelial cells and binding of laminin mediates differentiation and the production of capillary-like structures in vitro (Grant et al.). RGD domains are part of the cell adhesion sites of fibronectin and vitronectin (Ruoslahti et al., Humphries et al.).

The upper panel of FIG. 24 provides a comparison of the RGD domain in the KSHV Glycoprotein B protein sequence with other known RGD sequences. The residues flanking the RGD triplet show some similarity between the proteins. In particular, a number of the sequences have serine (S) and threonine (T) residues immediately flanking the RGD triplet, other T, S, F, and P residues to the C-terminal side, and a C residue in the N-terminal direction.

The lower panel of FIG. 24 shows an alignment of the KSHV Glycoprotein B protein sequence with the Glycoprotein B sequence of other gamma herpes viruses. Potential peptidase cleavage sites for the KSHV protein are indicated, based on the possession of cleavage sites in the other sequences. The RGD triplet is located about 3–9 residues from the expected N-terminus of the mature protein. There is no RGD sequence present in the Glycoprotein B of gamma herpes viruses outside the RFHV/KSHV subfamily. If the triplet mediates the infectivity or pathology of the KSHV virus, this property may be unique in comparison to viruses outside the subfamily.

The presence of an RGD domain at the N-terminus of the KSHV glycoprotein B suggests that the domain mediates attachment of KSHV to cells containing an appropriate integrin receptor, such as B-lymphocytes and endothelial cells, leading to infection of these cells. It is also possible that the domain mediates the differentiation of infected endothelial cells into capillary-like structures that are characteristic of Kaposi's sarcoma lesions. Blocking the attachment of KSHV to cells through the Glycoprotein B RGD domain may inhibit infection, tumor formation, or angiogenesis.

The RGD triplet in Glycoprotein B is potentially important in therapeutic approaches to KSHV infection in several respects. In one example, it may be of benefit to develop vaccines that are based on or enriched for Glycoprotein B peptides that incorporate the RGD sequence. Using a KSHV peptide of 7–20 amino acids encompassing this region, enough immunogenicity may be present to elicit antibodies for which the RGD would be part of the triplet. Circulating antibodies with this specificity may rapidly sequester the RGD site, and decrease any ability of this region to participate in viral infectivity or pathology.

In another example, peptides based on the KSHV Glycoprotein B sequence and including the RGD triplet may also inhibit viral infectivity or pathology, and could be administered immediately to counter an acute exposure. To the extent that binding to the RGD receptor also depends on residues in the ligand that neighbor the RGD triplet, the inhibition may be somewhat selective for KSHV virus in comparison with other RGD-bearing substances.

The full glycoprotein B sequence of RFHV is obtained by a similar strategy to that used for obtaining the KSHV sequence. The source for RFHV DNA is similarly prepared tissue from infected monkeys at the University of Washing-

ton Regional Primate Research Center. DNA is extracted as described in Example 5.

In order to obtain further sequence data in the 5' direction of the coding strand, amplifications are conducted using the consensus-degenerate (Type 1) oligonucleotide hybridizing upstream from the fragment as the 5' primer, in combination with the closest virus-specific (Type 3) oligonucleotides as the 3' primers. Thus, a first series of amplification cycles are conducted, for example, using FRFDA and AAITB as the first set of primers. This is followed by a second series of amplification cycles, conducted the same primers, or using the nested set FRFDA and GMTEB. Amplification conditions are similar to those described for KSHV.

In order to obtain further sequence data in the 3' direction of the coding strand, amplifications are conducted using consensus-degenerate (Type 1) oligonucleotides hybridizing downstream from the fragment as the 3' primer, in combination with the closest virus-specific (Type 3) oligonucleotides as the 5' primers. Thus, a first series of amplification cycles are conducted using NVFDB and VEGLA, followed by a second series conducted using NVFDB and PVLTA. Amplification and sequencing is performed as before. The new sequence is used to design further Type 3 oligonucleotides with a sense orientation, which are used with other downstream-hybridizing Type 1 oligonucleotides (namely FREYB and NVFDB) to obtain further sequence data.

Polynucleotide and amino acid sequence data is used to compare the Glycoprotein B of RFHV and KSHV with each other, and with that of other herpes viruses. The RFHV and KSHV sequences may be used to design further subfamily-specific Type 2 oligonucleotides, as in Example 6.

Example 8: Glycoprotein B sequences from DNA libraries

Complete Glycoprotein B sequences can be obtained or confirmed by generating DNA libraries from affected tissue. Sources of DNA for this study are the same as for Example 7.

The DNA lysate is digested with proteinase K, and DNA is extracted using phenol-chloroform. After extensive dialysis, the preparation is partially digested with the *Sau3A* I restriction endonuclease. The digest is centrifuged on a sucrose gradient, and fragments of about 10–23 kilobases are recovered. The *lambda* DASH-2™ vector phage (Stratagene) is prepared by cutting with *Bam*HI. The size-selected fragments are then mixed with the vector and ligated using DNA ligase.

The ligated vector is prepared with the packaging extract from Stratagene according to manufacturer's directions. It is used to infect XL1-BLUE™ MRA bacteria. About 200,000 of the phage-infected bacteria are plated onto agar at a density of about 20,000 per plate. After culturing, the plates are overlaid with nitrocellulose, and the nitrocellulose is cut into fragments. Phage are eluted from the fragments and their DNA are subjected to an amplification reaction using appropriate virus-specific primers. The reaction products are run on an agarose gel, and stained with ethidium bromide. Phage are recovered from regions of the plate giving amplified DNA of the expected size. The recovered phage are used to infect new XL1 bacteria and re-plated in fresh cultures. The process is repeated until single clones are obtained at limiting dilution.

Each clone selected by this procedure is then mapped using restriction nucleases to ascertain the size of the fragment incorporated. Inserts sufficiently large to incorporate the entire Glycoprotein B sequence are sequenced at

both ends using vector-specific primers. Sequences are compared with the known polynucleotide sequence of the entire EBV genome to determine whether the fragment spans the intact Glycoprotein B sequence. DNA is obtained from suitable clones, sheared, and sequenced by shot-gun cloning according to standard techniques.

Example 9: Antigenic regions of Glycoprotein B

The polynucleotide fragments between the hybridization sites for NIVPA and TVNCB in the Glycoprotein B gene have the predicted amino acid sequences shown in FIG. 14. Based on these sequences, peptides that are unique for RFHV or KSHV, or that are shared between species can be identified.

FIG. 14 shows example peptides of 6 or 7 amino acids in length. Some of the peptides comprise one or more residues that are distinct either for RFHV or KSHV (Class III), or for the RFHV/KSHV subfamily (Class II) compared with the corresponding gamma herpes virus peptides.

To confirm that regions contained within this 106-amino acid region of Glycoprotein B may be recognized by antibody, computer analysis was performed to generate Hopp and Woods antigenicity plots. The Hopp and Woods determination is based in part on the relative hydrophilicity and hydrophobicity of consecutive amino acid residues (Hopp et al).

Results are shown in FIGS. 20, 21 and 22. Key: ~ = antigenic; ^ = hydrophobic; # = potential N-linked glycosylation site. FIG. 20 shows the analysis of the 106 amino acid Glycoprotein B fragment from RFHV; FIG. 21 shows the analysis of the KSHV fragment, and FIG. 22 shows the analysis of the full KSHV sequence.

Both RFHV and KSHV contain several regions predicted to be likely antibody target sites. In particular, the KSHV sequence shows an antigenic region near the N-terminal end of this fragment, and near the potential N-linked glycosylation site. The full-length KSHV sequence shows hydrophobic minima corresponding both to the signal peptide (residue ~25) and the transmembrane domain (residue ~750). A number of putative antigenic regions with scores >1.0 or >1.5 are observed. Particularly notable is a region scoring up to ~2.5 that appears at about residues 440–460.

Example 10: Virus specific Glycoprotein B amplification assays

Type 3 oligonucleotides are used in nested virus-specific amplification reactions to detect the presence of RFHV or KSHV in a panel of tissue samples from potentially affected subjects.

For KSHV, DNA is extracted from tissue suspected of harboring the virus; particularly biopsy samples from human subjects with Kaposi's Sarcoma lesions and body cavity B-cell lymphoma. A number of different tissue samples are used, including some from KS lesions, some from apparently unaffected tissue in the same individuals, some from HIV positive individuals with no apparent KS lesions, and some from HIV negative individuals. Five samples are obtained in each category. DNA is prepared as described in Example 2.

The oligonucleotide primers GLTEA, YELPA, VNVNB, and ENTFB are ordered from Oligos Etc., Inc. The DNA is amplified in two stages, using primers GLTEA and ENTFB in the first stage, and YELPA and VNVNB in the second stage. The conditions of the amplification are similar to those of Example 3. The reaction product is electrophoresed

on a 2% agarose gel, stained with ethidium bromide, and examined under U.V. light. A positive result is indicated by the presence of abundant polynucleotide in the reaction product, as detected by ethidium bromide staining. This reflects the presence of KSHV derived DNA in the sample; specifically, the Glycoprotein B encoding fragment from YELPA to VNVNB. Results are matched with patient history and sample histopathology to determine whether positive assay results correlate with susceptibility to KS.

For RFHV, DNA is extracted from frozen tissue samples taken from *Macaca nemestrina* and *Macaca fascicularis* monkeys living in the primate colony at the Washington Regional Primate Research Center. Ten samples are taken each from tissue sites showing overt symptoms of fibromatosis, apparently unaffected sites in the same monkeys, and corresponding sites in monkeys showing no symptomatology. Nested PCR amplification is conducted first using GMTEA and VEGLB, then using KYEIA and TDRDB. Amplification product is electrophoresed and stained as before, to determine whether RFHV polynucleotide is present in the samples.

Example 11: Immunogenic regions of Glycoprotein B

To identify what antibodies may be generated during the natural course of infection with KSHV, serum samples are obtained from 10–20 AIDS subjects with Kaposi's Sarcoma lesions, from 10–20 HIV-positive symptom-negative subjects, and 10–20 HIV-negative controls. In initial studies, sera in each population are pooled for antibody analysis.

Peptides 12 residues long are synthesized according to the entire predicted extracellular domain of the mature KSHV Glycoprotein B molecule. Sequential peptides are prepared covering the entire sequence, and overlapping by 8 residues. The peptides are prepared on a nylon membrane support by standard F-Moc chemistry, using a SPOTS™ kit from Genosys according to manufacturer's directions. Prepared membranes are overlaid with the serum, washed, and overlaid with beta-galactose conjugated anti-human IgG. The test is developed by adding the substrate X-gal. Positive staining indicates IgG antibody reactivity in the serum against the corresponding peptide.

Similarly, to identify antibodies formed in the natural course of RFHV infection, blood samples are collected from 10 *Macaca nemestrina* and 10 *Macaca fascicularis* monkeys, a proportion of which display overt symptoms of fibromatosis. The presence or absence of an ongoing RFHV infection is confirmed by conducting PCR amplification assays using RFHV-specific oligonucleotides as in Example 10. Plasma and blood cells are separated by centrifugation. These sera are used to test for antibodies in a method similar to that for KSHV, except that 12-mers are synthesized based on the RFHV Glycoprotein B sequence.

Select RFHV and KSHV peptides are also tested in animal models to determine immunogenicity when administered in combination with desirable adjuvants such as alum and DETOX™. Suitable peptides include those identified in the aforementioned experiment as eliciting antibody during the natural course of viral infection. Other candidates include those believed to participate in a biological function of Glycoprotein B, and those corresponding to peptides of other herpes viruses known to elicit viral neutralizing antibodies. The peptides are coupled onto keyhole limpet hemocyanin (KLH) as a carrier, combined with adjuvant according to standard protocols, and 100 µg peptide equivalent in 1–2 mL inoculum is injected intramuscularly into

rabbits. The animals are boosted with a second dose 4 weeks later, and test-bled after a further 2 weeks.

Microtiter plate wells are prepared for ELISA by coating with the immunogen or unrelated peptide-KLH control. The wells are overlaid with serial dilutions of the plasma from the test bleeds, washed, and developed using beta-galactose anti-human IgG and X-gal. Positive staining in the test wells but not the control wells indicates that the peptide is immunogenic under the conditions used.

Example 12: Identification and characterization of Glycoprotein B from other members of the RFHV/KSHV subfamily

Tissue samples suspected of containing a previously undescribed gamma herpes virus, particularly fibroproliferative conditions, lymphocyte malignancies, and conditions associated with immunodeficiency and immunosuppression, such as acute respiratory disease syndrome (ARDS), are preserved by freezing, and the DNA is extracted as in Example 2. Two rounds of PCR amplification are conducted using Type 1 oligonucleotides FRFDA and TVNCB in the first round, then using nested Type 1 or Type 2 oligonucleotides in the second round.

Optionally, the presence of an RFHV/KSHV family Glycoprotein B polynucleotide is confirmed by probing the amplification product with a suitable probe. The amplified polynucleotide is electrophoresed in agarose and blotted onto a nylon membrane. The blot is hybridized with a probe comprising the polynucleotide fragment obtained from the KSHV polynucleotide encoding Glycoprotein B (residues 36–354 of SEQ. ID NO:3), labeled with ³²P. The hybridization reaction is done under conditions that will permit a stable complex forming between the probe and Glycoprotein B from a herpes virus, but not between the probe and Glycoprotein B encoding polynucleotides from sources outside the RFHV/KSHV subfamily. Hybridization conditions will require approximately 70% identity between hybridizing segments of the probe and the target for a stable complex to form. These conditions are calculated using the formula given earlier, depending on the length and sequence of the probe and the corresponding sequence of the target. The conditions are estimated to be: a) allowing the probe to hybridize with the target in 6×SSC (0.15M NaCl, 15 mM sodium citrate buffer) at room temperature in the absence of formamide; and b) washing newly formed duplexes for a brief period (5–10 min) in 2×SSC at room temperature.

Amplified polynucleotides that hybridize to the labeled probe under these conditions are selected for further characterization. Alternatively, PCR amplification products having about the same size as that predicted from the KSHV are suspected of having a related sequence. Samples may also be suspected of having a related sequence if they have been used to obtain polynucleotides encompassing other regions of a herpes virus genome, such as DNA polymerase. Samples containing fragments potentially different from RFHV or KSHV, either due to a size difference or different origin, are sequenced across the fragment as in Example 4. Those with novel sequences are used to determine the entire Glycoprotein B gene sequence by a method similar to that in Example 7 or 8.

A Glycoprotein B encoding sequence from a third member of the RFHV/KSHV herpes virus subfamily was obtained as follows.

DNA was extracted from two frozen tissue samples from a *Macaca mulatta* monkey with retroperitoneal fibromatosis. Extraction was conducted according to Example 1. The

extracted DNA was precipitated with ethanol in the presence of 40 μ g glycogen as carrier, washed in 70% ethanol, and resuspended in 10 mM Tris buffer, pH 8.0. The extracted DNA was used to obtain a 151 base pair fragment of a herpes virus DNA polymerase gene, which was non-identical to that of KSHV, RFHV, or any other previously characterized DNA polymerase. This led to the suspicion that the sample contained genomic DNA from a different herpes virus, that could be used to identify and characterize a new Glycoprotein B gene.

A 386 base pair fragment of a Glycoprotein B encoding sequence was amplified from the sample using a hemi-nested PCR. The procedure was similar to that used in Examples 4 and 5, with a first round of amplification using FRFDA and TVNCB, followed by a second round of amplification using NIVPA and TVNCB. The final PCR product was sequenced as before.

FIG. 23 lists the polynucleotide sequence (SEQ. ID NO:96) along with the corresponding amino acid translation (SEQ. ID NO:97). Underlined is the 319 base pair sequence in between the two primer hybridization sites. The sequences are different from those of KSHV and RFHV. The Glycoprotein B is from a new member of the RFHV/KSHV subfamily of herpes viruses, designated RFHV2.

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SEQUENCES

SEQ. ID	Designation	Description	Type	Source
1	RFHV	Glycoprotein B PCR segment	dsDNA	FIG. 1
2	RFHV	Glycoprotein B PCR segment	Protein	FIG. 1
3	KSHV	Glycoprotein B PCR segment	dsDNA	FIG. 1
4	KSHV	Glycoprotein B PCR segment	Protein	FIG. 1
5	sHV1	Glycoprotein B sequence	dSDNA	GenBank
6	bHV4	Glycoprotein B sequence	dsDNA	HSVSPOLGBP
7	eHV2	Glycoprotein B sequence	dsDNA	GenBank
8	mHV68	Glycoprotein B sequence	dsDNA	EHVU20824
9	hEBV	Glycoprotein B sequence	dsDNA	GenBank
10	hCMV	Glycoprotein B sequence	dsDNA	EBV
11	hHV6	Glycoprotein B sequence	dsDNA	GenBank
12	hVZV	Glycoprotein B sequence	dsDNA	HEH6GBXA
13	HSV1	Glycoprotein B sequence	dsDNA	HEVZVXX
14	sHV1	Glycoprotein B sequence	Protein	GenBank
15	bHV4	Glycoprotein B sequence	Protein	Translation
16	eHV2	Glycoprotein B sequence	Protein	Translation
17	mHV68	Glycoprotein B sequence	Protein	Translation
18	hEBV	Glycoprotein B sequence	Protein	Translation
19	hCMV	Glycoprotein B sequence	Protein	Translation
20	hHV6	Glycoprotein B sequence	Protein	Translation
21	hVZV	Glycoprotein B sequence	Protein	Translation
22	HSV1	Glycoprotein B sequence	Protein	Translation
23	sHVSAs	Glycoprotein B sequence	Protein	Translation
24–40		TYPE 1 oligonucleotides (Gamma herpes Glycoprotein B)	ssDNA	Table 4
41–47		TYPE 2 oligonucleotide (RFHV/KSHV subfamily Glycoprotein B)	ssDNA	Table 6
48–55		TYPE 3 oligonucleotides - RFHV specific Glycoprotein B	ssDNA	Table 7
56–63		TYPE 3 oligonucleotides - KSHV specific Glycoprotein B	ssDNA	Table 7
64–66		CLASS I antigen peptides (Gamma herpes Glycoprotein B)	Protein	Table 8
67–72		CLASS II antigen peptides (RFHVIKSHV subfamily Glycoprotein B)	Protein	Table 8
73–74		CLASS III antigen peptides - RFHV specific Glycoprotein B	Protein	Table 8

-continued

SEQUENCES				
SEQ. ID	Designation	Description	Type	Source
75-76		CLASS III antigen peptide s-KSHV specific Glycoprotein B	Protein	Table 8
77-78		TYPE 1 oligonucleotide (Gamma herpes Capsid maturation)	ssDNA (IUPAC)	Table 9
79		TYPE 1 oligonucleotide (Gamma herpes DNA polymerase)	ssDNA (IUPAC)	Table 9
80-87		TYPE 3 oligonucleotides - KSHV specific Glycoprotein B		Table 11
88-90		TYPE 3 oligonucleotides - KSHV specific DNA Polymerase		Table 11
91	KSHV	DNA sequence comprising encoding regions for Capsid Maturation fragment, Glycoprotein B, and DNA polymerase fragment	dsDNA	FIG. 18
92	KSHV	DNA sequence comprising encoding regions for Capsid Maturation fragment and Glycoprotein B (residues 1-3056)	dsDNA	Example 7
93	KSHV	Capsid Maturation sequence	Protein	FIG. 18
94	KSHV	Glycoprotein B sequence	Protein	FIG. 18
95	KSHV	DNA polymerase sequence	Protein	FIG. 18
96	RFHV2	Glycoprotein B PCR segment	dsDNA	FIG. 22
97	RFHV2	Glycoprotein B PCR segment	Protein	FIG. 22
98		Shared sequence	dsDNA	Example 7
99-100		CLASS I antigen peptides of Glycoprotein B	Protein	Table 8
101-105		Signal peptidase cleavage regions	Protein	FIG. 24
106-113		Peptide sequences comprising RGD domains	Protein	FIG. 24

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 113

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 386 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GTGTACAAGA AGAACATCGT GCCGTACATT TTCAAGGTAC GCAGGTACAT AAAAATAGCA      60
ACATCTGTCA CGGTCTACCG CGGTATGACA GAAGCAGCAA TCACAAACAA ATATGAGATC      120
CCCAGGCCCG TGCCTCTCTA CGAGATCAGT CACATGGACA GCACCTACCA GTGCTTTAGT      180
TCCATGAAAA TTGTAGTGAA CGGAGTCGAA AATACGTTCA CCGATCGGGA TGACGTAAAC      240
AAAACCGTAT TTCTCCAGCC CGTCGAAGGT CTAAGTGACA ACATACAAAG ATACTTTAGC      300
CAACCAGTAC TGTACTCTGA ACCCGGATGG TTCCCAGGTA TCTACAGGGT TGGGACAACA      360
GTAAACTGTG AGATTGTAGA CATGTT                                     386

```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 128 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Val Tyr Lys Lys Asn Ile Val Pro Tyr Ile Phe Lys Val Arg Arg Tyr
1           5           10           15

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-continued

Ile Lys Ile Ala Thr Ser Val Thr Val Tyr Arg Gly Met Thr Glu Ala
 20 25 30

Ala Ile Thr Asn Lys Tyr Glu Ile Pro Arg Pro Val Pro Leu Tyr Glu
 35 40 45

Ile Ser His Met Asp Ser Thr Tyr Gln Cys Phe Ser Ser Met Lys Ile
 50 55 60

Val Val Asn Gly Val Glu Asn Thr Phe Thr Asp Arg Asp Asp Val Asn
 65 70 75 80

Lys Thr Val Phe Leu Gln Pro Val Glu Gly Leu Thr Asp Asn Ile Gln
 85 90 95

Arg Tyr Phe Ser Gln Pro Val Leu Tyr Ser Glu Pro Gly Trp Phe Pro
 100 105 110

Gly Ile Tyr Arg Val Gly Thr Thr Val Asn Cys Glu Ile Val Asp Met
 115 120 125

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 386 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTGTACAAGA AGAACATCGT GCCGTATATT TTTAAGGTGC GCGCGTATAG GAAAATTGCC 60

ACCTCTGTCA CGGTCTACAG GGGCTTGACA GAGTCCGCCA TCACCAACAA GTATGAACTC 120

CCGAGACCCG TGCCACTCTA TGAGATAAGC CACATGGACA GCACCTATCA GTGCTTTAGT 180

TCCATGAAGG TAAATGTCAA CGGGGTAGAA AACACATTTA CTGACAGAGA CGATGTTAAC 240

ACCACAGTAT TCCTCCAACC AGTAGAGGGG CTTACGGATA ACATTCAAAG GTACTTTAGC 300

CAGCCGGTCA TCTACGCGGA ACCCGGCTGG TTTCCCGGCA TATACAGAGT TAGGACAACA 360

GTCAACTGTG AGATTGTAGA CATGTT 386

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 128 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Val Tyr Lys Lys Asn Ile Val Pro Tyr Ile Phe Lys Val Arg Arg Tyr
 1 5 10 15

Arg Lys Ile Ala Thr Ser Val Thr Val Tyr Arg Gly Leu Thr Glu Ser
 20 25 30

Ala Ile Thr Asn Lys Tyr Glu Leu Pro Arg Pro Val Pro Leu Tyr Glu
 35 40 45

Ile Ser His Met Asp Ser Thr Tyr Gln Cys Phe Ser Ser Met Lys Val
 50 55 60

Asn Val Asn Gly Val Glu Asn Thr Phe Thr Asp Arg Asp Asp Val Asn
 65 70 75 80

Thr Thr Val Phe Leu Gln Pro Val Glu Gly Leu Thr Asp Asn Ile Gln
 85 90 95

Arg Tyr Phe Ser Gln Pro Val Ile Tyr Ala Glu Pro Gly Trp Phe Pro
 100 105 110

-continued

Gly Ile Tyr Arg Val Arg Thr Thr Val Asn Cys Glu Ile Val Asp Met
 115 120 125

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2425 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATGGTACCTA ATAAACACTT ACTGCTTATA ATTTGTTCGT TTTCTACTGC ATGTGGACAA 60
 ACGACACCTA CTACAGCTGT TGAATAAAT AAAACTCAAG CTATATACCA AGAGTATTTC 120
 AAATATCGTG TATGTAGTGC ATCAACTACT GGAGAATTGT TTAGATTGA TTTAGACAGA 180
 ACTTGTCCTA GTACTGAAGA CAAAGTTCAT AAGGAAGGCA TTCTTTTAGT GTACAAAAAA 240
 AATATAGTTC CATATATCTT TAAAGTCAGA AGATACAAAA AAATCACAAAC ATCAGTCCGT 300
 ATTTTAAATG GCTGGACTAG AGAAGGTGTT GCTATTACAA ACAAATGGGA ACTTTCTAGA 360
 GCTGTTCCAA AATATGAGAT AGATATTATG GATAAGACTT ACCAATGTCA TAATTGCATG 420
 CAGATAGAAG TAAACGGAAT GTTAAATTCT TACTATGACA GAGATGGAAA TAACAAAACCT 480
 GTAGACTTAA AGCCTGTAGA TGGTCTAACG GGTGCAATTA CAAGATACAT TAGCCAACCT 540
 AAAGTTTTTG CTGATCCTGG CTGGCTATGG GGAACCTTACA GGACTCGAAC TACCGTTAAC 600
 TGTGAAATTG TAGACATGTT TGCTAGGTCT GCTGACCCTT ACACATACTT TGTGACTGCG 660
 CTGCGGACA CAGTAGAAGT GTCTCCTTTC TGTGATGTAG ATAATTCATG CCCAAATGCA 720
 ACTGACGTGT TGTCACTACA AATAGACTTA AATCACACTG TTGTTGACTA TGGAAATAGA 780
 GCTACATCAC AGCAGCATAA AAAAGAATA TTTGCTCATA CTTTAGATTA TTCTGTTTCT 840
 TGGGAAGCTG TAAACAAATC CGCGTCAGTA TGCTCAATGG TTTTGTGGAA GAGTTTTCOA 900
 CGAGCTATCC AAATGAACA TGAATTAACT TATCATTTC AATGCTAATGA AATAACAGCA 960
 GGATTCTCTA CAGTGAAAGA ACCCTTAGCA AATTTTACAA GTGATTACAA TTGTCTTATG 1020
 ACTCATATCA AACTACTTTT AGAGGATAAG ATAGCAAGAG TCAACAATAC TCACACTCCA 1080
 AATGGTACAG CAGAATATTA TCAACAGAA GGTGGAATGA TTTTAGTGTG GCAGCCATTA 1140
 ATAGCAATAG AATTAGAAGA AGCAATGTTG GAAGCAACTA CATCTCCAGT AACTCCTAGT 1200
 GCACCAACTA GCTCATCTAG AAGTAAGCGA GCAATAAGAA GCATAAGAGA TGTGAGTGCA 1260
 GGTTCAGAAA ATAATGTGTT TCTATCACAA ATACAATATG CATATGATAA GCTACGTCAA 1320
 AGTATCAACA ACGTGCTAGA AGAGTTAGCT ATAACATGGT GTAGAGAACA AGTGAGACAA 1380
 ACAATGGTGT GGTATGAGAT AGCAAAAATT AATCCAACAA GTGTTATGAC AGCAATATAT 1440
 GGAAAACCTG TCTCTCGTAA AGCTTTAGGA GATGTAATCT CTGTTACAGA ATGTATAAAT 1500
 GTTGACCAAT CTAGTGTGAG CATAACAAG AGTCTTAAAA CAGAAAATAA TGACATATGC 1560
 TATTCACGGC CTCCAGTTAC ATTTAAATTT GTTAACAGTA GTCAGCTGTT TAAAGGACAG 1620
 TTAGGGGCTA GAAATGAAAT TCTTCTGTCA GAAAGTCTTG TAGAAAATTG CCACCAAAAT 1680
 GCAGAGACTT TTTTACAGC TAAAAATGAA ACTTACCCTT TAAAAATTA TGTGCATGTA 1740
 GAAACTTTGC CAGTGAATAA CATTTCAACT TTAGACACTT TTTTAGCTCT TAACCTAACT 1800
 TTCATAGAAA ATATGACTT TAAAGCTGTT GAATTGTATT CAAGTGGAGA GAGAAAGTTA 1860
 GCAAACGTGT TTGATTTAGA GACTATGTTT AGAGAATATA ACTATTACGC TCAGAGTATA 1920
 TCTGGCTTAA GAAAAGATTT TGATAACTCT CAAAGAAACA ACAGAGACAG AATCATTTCAA 1980

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GATTTTTCAG	AAATTC TAGC	AGACTTAGGC	TCTATCGGCA	AAGTTATTGT	TAATGTGGCA	2040
AGCGGCGCAT	TTTCTCTTTT	TGGAGGTATT	GTAACAGGCA	TATTAAATTT	TATTA AAAAT	2100
CCTTTAGGTG	GCATGTTTAC	ATTTCTATTA	ATAGGAGCAG	TTATAATCTT	AGTAATTCTA	2160
CTAGTACGGC	GCACAAATAA	TATGTCTCAA	GCTCCAATTA	GAATGATTTA	CCCAGATGTT	2220
GAGAAATCTA	AATCTACTGT	GACGCCTATG	GAGCCTGAAA	CAATTAAACA	AATTTTGCTT	2280
GGAATGCATA	ACATGCAGCA	AGAAGCATAT	AAGAAAAAAG	AAGAACAAAG	AGCTGCTAGA	2340
CCGTCTATTT	TTAGACAAGC	TGCTGAGACA	TTTTTGCGTA	AGCGATCTGG	TTACAAACAG	2400
ATTTC AACCG	AAGACAAAAT	AGTAT				2425

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 2623 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATGTATTATA	AGACTATCTT	ATTCTTCGCT	CTAATTAAGG	TATGCAGTTT	CAACCAGACC	60
ACTACACACT	CAACCACAAC	CTCACCAAGT	ATTTCATCAA	CCACCTCTTC	CACAACAACA	120
TCAACAAGCA	AGCCATCAAA	CACAACCTCA	ACAAATAGTT	CATTAGCTGC	CTCTCCCCAG	180
AACACGTCAA	CAAGCAAGCC	ATCCACTGAT	AATCAGGGTA	CCAGTACCCC	CACTATTCCA	240
ACTGTTACTG	ATGACACAGC	CAGTAAAAAT	TTTATAAAAT	ACAGAGTATG	CAGTGCATCA	300
TCTTCTCTG	GAGAACTATT	CAGATTTGAC	CTTGATCAGA	CATGTCCAGA	TACAAAAGAT	360
AAAAAACATG	TGGAAGGCAT	CCTGCTGGTA	CTAAAAAAGA	ATATTGTCCC	ATACATCTTC	420
AAAGTGAGGA	AATATAGAAA	AATTGCCACC	TCAGTGACAG	TTTACAGAGG	GTGGTCCCAG	480
GCAGCTGTTA	CCAATAGGGA	TGATATCAGC	AGAGCCATAC	CCTATAATGA	AATTTCAATG	540
ATAGATAGGA	CCTATCATTT	TTTCTCTGCT	ATGGCAACAG	TCATTAATGG	GATTCTGAAC	600
ACCTATATAG	ACAGGGATTC	TGAAAATAAG	TCTGTTCCCC	TCCAGCCAGT	GGCCGGA CTG	660
ACTGAGAACA	TAAACAGATA	CTTTAGTCAA	CCTCTCATAT	ATGCAGAACC	TGGCTGGTTT	720
CCAGGGATTT	ATAGAGTGAG	AACAAC TGTT	AATTGTGAGG	TTGTTGACAT	GTATGCCCCG	780
TCTGTGGAAC	CATATACTCA	CTTTATTACA	GCTCTGGGGG	ACACTATTGA	AATCTCCCCA	840
TTCTGT CACA	ACAATTCTCA	ATGCACCACT	GGTAATTCCA	CCTCAAGGGA	TGCCACAAAG	900
GTATGGATAG	AAGAAAATCA	CCAACTGTT	GACTATGAAA	GACGGGGGCA	TCCCACTAAA	960
GATAAAGAA	TCTTTCTAAA	AGATGAGGAA	TATACCATCT	CCTGGAAAGC	AGAAGATAGA	1020
GAGAGAGCTA	TTTGTGATTT	TGTGATATGG	AAAACCTTTC	CCAGGGCCAT	ACAAACAATC	1080
CATAATGAGA	GCTTTCAC TT	TGTGGCAAAT	GAAGTCACAG	CCAGCTTTTT	AACATCCAAC	1140
CAAGAAGAAA	CGGAGCTACG	TGGAAATACC	GAGATATTGA	ATTGCATGAA	TAGTACCATA	1200
AATGAAACTC	TAGAAGAGAC	AGTCAAAAAA	TTTAACAAAT	CCCATATCAG	AGATGGGGAG	1260
GTAAAGTACT	ATAAAACAAA	TGGGGGACTA	TTCTTATCT	GGCAGGCAAT	GAAACCCCTT	1320
AATCTGTCAG	AACACACAAA	CTACACTATT	GAAAGGAATA	ACAAGACTGG	AAATAAATCA	1380
AGACAAAAAA	GGTCTGTAGA	TACAAAGACC	TTCCAAGGCG	CCAAGGGCCT	GTCCACTGCC	1440
CAGGTTCAAT	ATGCCTATGA	CCATTTAAGA	ACAAGCATGA	ATCACATCCT	AGAGGAATTA	1500
ACCAAAACAT	GGTGCCGGGA	ACAAAAAAG	GACAATCTAA	TGTGGTATGA	GCTGAGTAAA	1560

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ATTAACCCAG	TGAGTGTTCAT	GGCAGCCATT	TATGGGAAAC	CTGTGGCAGT	GAAAGCCATG	1620
GGAGATGCAT	TCATGGTTTC	TGAGTGCATC	AATGTTGACC	AGGCAAGTGT	CAATATCCAT	1680
AAAAGTATGA	GAACGGATGA	TCCCAAGGTA	TGTTACTCCA	GACCCCTGGT	CACATTTAAA	1740
TTTGTGAATA	GTACTGCCAC	CTTCAGGGGT	CAGCTTGGAA	CAAGGAATGA	AATCTTGCTC	1800
ACAAACACAC	ACGTGGAAC	TTGTAGACCA	ACAGCAGATC	ATTATTTTTT	TGTAAAGAAC	1860
ATGACACACT	ATTTTAAGGA	CTATAAATTT	GTGAAGACAA	TGGATACCAA	TAACATATCC	1920
ACCCTGGATA	CATTTTAAAC	TCTCAATTTA	ACTTTTATAG	ACAATATAGA	TTTCAAGACA	1980
GTGGAACCTT	ACAGTGAGAC	TGAAAGAAAG	ATGGCCAGTG	CCCTCGACCT	GGAGACGATG	2040
TTTAGAGAGT	ATAATTACTA	CACACAGAAG	CTTGCAAGTC	TGAGAGAAGA	TCTAGACAAC	2100
ACCATTGACC	TGAACAGGGA	CAGACTAGTT	AAAGATCTCT	CTGAAATGAT	GGCAGACCTT	2160
GGAGACATTG	GAAAAGTGGT	GGTCAACACA	TTCAGTGGCA	TTGTCACTGT	TTTGGGTCT	2220
ATAGTTGGTG	GATTGTGTCAG	TTTTTTCACA	AACCCCATTG	GGGGCGTGAC	GATCATCCTC	2280
CTTCTCATAG	TTGTGGTTTT	TGTTGTTTTT	ATAGTCTCCA	GGAGAACCAA	TAACATGAAC	2340
GAGGCCCCCA	TAAAAATGAT	CTATCCAAAC	ATTGACAAAG	CCTCTGAGCA	GGAGAACATT	2400
CAGCCCTTAC	CCGGAGAGGA	GATTAAGCGC	ATCCTCCTTG	GAATGCACCA	GCTCCAGCAA	2460
AGTGAGCAGC	GCAAATCTGA	GGAAGAGGCT	AGCCATAAAC	CAGGGTTGTT	CCAACTATTG	2520
GGGGATGGCC	TACAATTGCT	GCGCAGGCGC	GGGTATACTA	GGTTACCAAC	TTTGTACCCC	2580
AGTCCAGGCA	ATGACACATC	TGAGACACAC	CAAAAATATG	TTT		2623

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 2625 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATGGGGGTGCG	GGGGCGGGCC	TCGCGTCGTC	CTCTGTCTAT	GGTGCCTGCG	TGCGCTTCTC	60
TGCCAGGGGG	TGGCGCAAGA	AGTTGTGGCT	GAAACGACCA	CCCCGTTGCG	AACCCACAGA	120
CCAGAAGTGG	TGGCCGAGGA	GAACCCGGCC	AACCCCTTTC	TGCCGTTTCT	GGTATGCGGG	180
GCCTCGCCTA	CGGGCGGAGA	GATATTCAGG	TTCCCCCTGG	AGGAGAGCTG	CCCCAACACG	240
GAAGACAAGG	ACCACATAGA	GGGCATAGCT	CTCATCTACA	AGACCAACAT	AGTGCCTTAT	300
GTTTTTAATG	TCAGAAAGTA	TAGGAAGATC	ATGACCTCGA	CCACCATCTA	CAAGGGTTGG	360
AGCGAGGATG	CCATAACAAA	CCAGCACACG	AGGAGCTACG	CCGTCCCCCT	GTACGAGGTC	420
CAGATGATGG	ACCACTATTA	TCAGTGCTTT	AGCGCCGTAC	AGGTCAACGA	GGGGGGGCAC	480
GTCAACACCT	ACTATGACAG	GGACGGGTGG	AACGAGACCG	CCTTCCTCAA	ACCGGCCGAT	540
GGTCTCACCT	CTAGCATAAC	GCGCTATCAG	AGTCAACCAG	AGGTGTACGC	CACCCCCAGA	600
AACCTGTTGT	GGTCTTACAC	AACAAGAACC	ACAGTCAACT	GCGAGGTGAC	AGAGATGTCT	660
GCGAGATCCA	TGAAACCATT	TGAGTTCTTT	GTGACGTCTG	TTGGTGACAC	TATAGAGATG	720
TCGCCCTTTT	TAAAAGAAAA	TGGCACAGAG	CCAGAGAAAA	TCTTGAAAAG	ACCACACTCT	780
ATTCAACTGC	TGAAAACTA	TGCTGTACAC	AAGTACGGTG	TGGGGTTGGG	GCAGGCTGAT	840
AACGCTACCA	GATTCTTTGC	AATATTGGG	GACTATTCCC	TGTCTTGAA	AGCCACCACT	900
GAAAACAGCT	CCTACTGTGA	TTTAATTTTA	TGGAAGGGGT	TTTCCAATGC	CATTCAAACCT	960
CAACACAATA	GCAGTCTCCA	TTTTATTGCC	AATGATATAA	CAGCCTCCTT	CTCTACTCCT	1020

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TTAGAAGAAG	AGGCTAATTT	TAACGAGACA	TTTAAGTGTA	TATGGAACAA	CACCCAAGAA	1080
GAAATTCAAA	AAAAGTTAAA	AGAGGTTGAA	AAAACTCACA	GACCTAACGG	TACTGCGAAG	1140
GTCTATAAAA	CAACAGGCAA	TCTGTACATT	GTTTGGCAAC	CGCTTATACA	GATAGACCTG	1200
CTAGATACTC	ATGCCAAGCT	GTACAATCTC	ACAAACGCTA	CAGCTTCACC	TACATCAACA	1260
CCCACAACAT	CTCCCAGGAG	AAGACGCAGG	GATACTTCAA	GTGTTAGTGG	CGGTGGAAT	1320
AATGGAGACA	ACTCAACTAA	GGAAGAGAGT	GTGGCGGCCT	CCCAGGTTCA	GTTTGCCTAT	1380
GACAATCTCA	GAAAGAGCAT	CAACAGGGTG	TTGGGAGAGC	TGTCCAGGGC	ATGGTGCAGG	1440
GAACAGTACA	GGGCCTCGCT	CATGTGGTAC	GAGCTGAGCA	AGATCAACCC	CACCAGCGTC	1500
ATGAGCGCCA	TCTATGGCAG	GCCAGTGTCT	GCCAAGTTGA	TAGGGGACGT	GGTGTCACTG	1560
TCAGATTGTA	TCAGTGTGTA	CCAAAAGAGC	GTGTTTGTGC	ACAAAAATAT	GAAGGTGCCT	1620
GGCAAAGAAG	ACCTGTGTTA	CACCAGGCCT	GTGGTGGGCT	TCAAGTTTAT	CAATGGGAGC	1680
GAAGTGTGTTG	CTGGCCAGCT	GGGTCCCAGG	AACGAGATTG	TGCTGTCCAC	CTCTCAGGTG	1740
GAGGTCTGCC	AGCACAGCTG	CGAGCACTAC	TTCCAGGCCG	GGAACCAGAT	GTACAAGTAC	1800
AAGGACTACT	ACTATGTCAG	TACCCCTAAC	CTGACTGACA	TACCCACCCT	ACACACCATG	1860
ATTACCTCTGA	ACCTGTCTCT	GGTAGAGAAT	ATAGATTTTA	AGGTGATTGA	GCTCTATTCT	1920
AAAACAGAGA	AAAGGCTGTC	CAACGTGTTT	GACATCGAGA	CCATGTTCAG	GGAGTACAAC	1980
TACTACACTC	AGAACCTCAA	CGGGCTGAGG	AAGGACCTGG	ATGACAGCAT	AGATCATGGC	2040
AGGGACAGCT	TCATCCAGAC	CCTGGGTGAC	ATCATGCAGG	ACCTGGGCAC	CATAGGCAAG	2100
GTGGTGGTCA	ATGTGGCCAG	CGGAGTGTTC	TCCCTCTTTG	GGAGCATAGT	CTCGGGGGTG	2160
ATAAGCTTTT	TCAAAAATCC	CTTTGGGGGC	ATGCTGCTCA	TAGTCCTCAT	CATAGCCGGG	2220
GTAGTGGTGG	TGTACCTGTT	TATGACCAGG	TCCAGGAGCA	TATACTCTGC	CCCCATTAGA	2280
ATGCTCTACC	CCGGGGTGGA	GAGGGCGGCC	CAGGAGCCGG	GCGCGCACCC	GGTGTCAAG	2340
GACCAAATCA	GGAACATCCT	GATGGGAATG	CACCAATTTC	AGCAGCGGCA	GCGGGCGGAA	2400
GAGGAGGCCC	GACGAGAGGA	AGAAGTAAAA	GGAAAAAGAA	CTCTCTTTGA	AGTGATAAGA	2460
GAAGTCTGCGA	CCAGCGTTCT	GAGGAGGAGA	AGAGGGGGTG	GTGGGTACCA	GCGCCTACAG	2520
CGAGACGGGA	GCGACGATGA	GGGGGATTAT	GAGCCATTGA	GGCGACAAGA	TGGAGGCTAC	2580
GACGACGTGG	ACGTGGAGGC	AGGCACGGCG	GATACCGGTG	TGTAA		2625

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 2548 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATGTACCCTA	CAGTGAAGAAG	TATGAGAGTC	GCCCACCTAA	CCAATCTCCT	AACCCCTTCTG	60
TGTCTGCTGT	GCCACACGCA	TCTCTACGTA	TGTCAGCCAA	CCACTCTGAG	GCAGCCATCA	120
GACATGACCC	CAGCCCAGGA	CGCTCCAACA	GAGACTCCCC	CACCCCTCTC	AACTAACACT	180
AACAGAGGAT	TTGAGTACTT	TCGCGTGTGT	GGGGTGGCTG	CCACGGGGGA	GACCTTCAGG	240
TTTGATTAG	ACAAAACATG	CCCCAGTACA	CAAGATAAGA	AGCATGTGGA	GGGCATCTTG	300
CTCGTGTATA	AGATCAACAT	CGTGCCCTAC	ATCTTCAAAA	TCAGGAGATA	TAGAAAAATA	360
ATTACTCAAC	TGACCATCTG	GCGAGGCCTA	ACCACTAGTT	CAGTCACTGG	TAAATTTGAA	420

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ATGGCCACTC	AGGCCACGA	GTGGGAAGTG	GGCGACTTTG	ACAGCATCTA	TCAGTGCTAC	480
AATAGCGCCA	CCATGGTGGT	AAACAACGTC	AGACAGGTGT	ATGTGGACAG	AGATGGGGTC	540
AATAAACTG	TGAACATACG	CCCTGTTGAT	GGTCTAACAG	GGAATATCCA	AAGATACTTT	600
AGTCAGCCCA	CCCTTTATTC	AGAACCTGGT	TGGATGCCCTG	GCTTTTATCG	TGTTCGAACC	660
ACCGTTAACT	GTGAAATTGT	AGACATGGTG	GCACGCTCCA	TGGATCCCTA	TAACACATC	720
GCTACCGCCC	TGGGAGACAG	CCTGGAGCTC	TCCCCGTTTC	AAACCTTTGA	CAACACCAGC	780
CAGTGTACTG	CGCCTAAGAG	AGCTGATATG	AGGGTCAGGG	AGGTCAAGAA	TTACAAGTTT	840
GTAGATTATA	ATAACAGGGG	AACTGCCCCC	GCTGGACAAA	GCAGGACCTT	TCTAGAGACT	900
CCCTCTGCCA	CTTACTCCTG	GAAAACAGCC	ACCAGACAAA	CTGCCACGTG	CGACCTGGTG	960
CACTGGAAAA	CATTCCCCTG	CGCCATCCAA	ACTGCTCATG	AACATAGCTA	CCATTTTGTG	1020
GCCAATGAAG	TCACCGCCAC	CTTCAATACA	CCCCTGACTG	AGGTAGAAAA	TTTCACCAGC	1080
ACGTATAGCT	GCGTCAGTGA	CCAGATCAAT	AAGACCATCT	CTGAATATAT	CCAAAAGTTG	1140
AACAACCTCT	ACGTGGCCAG	TGGGAAAACA	CAGTATTTCA	AGACTGATGG	TAACCTGTAC	1200
CTCATCTGGC	AACCACTCGA	ACATCCAGAG	ATTGAAGACA	TAGACGAGGA	CAGCGACCCA	1260
GAACCAACCC	CCGCCCCACC	AAAGTCCACA	AGGAGAAAAA	GAGAGGCAGC	TGACAATGGA	1320
AACTCAACAT	CTGAGGTCTC	AAAGGGCTCA	GAAAATCCGC	TCATTACGGC	CCAAATTCAA	1380
TTTGCCATATG	ACAAGCTGAC	CACCAGCGTC	AACAACGTGC	TTGAGGAGTT	GTCCAGGGCG	1440
TGGTGTAGAG	AACAGGTCAG	AGACACCCTC	ATGTGGTATG	AGCTTAGCAA	GGTCAACCTT	1500
ACGAGTGTGA	TGTCTGCCAT	TTATGGAAG	CCTGTCGCTG	CCAGGTACGT	GGGCGACGCC	1560
ATATCTGTGA	CAGACTGTAT	CTATGTGGAC	CAAAGTTCAG	TCAACATCCA	CCAGAGCTTG	1620
CGGCTGCAGC	ATGATAAAAC	CACCTGCTAC	TCGAGACCTA	GAGTCACCTT	CAAAATTTATA	1680
AACAGTACAG	ACCCGCTAAC	TGGCCAGTTG	GGTCCTAGAA	AAGAAATTAT	CCTCTCCAAC	1740
ACAAACATAG	AAACATGCAA	GGATGAGAGT	GAACACTACT	TCATTGTGGG	GGAATACATT	1800
TACTATTATA	AAAATTACAT	TTTTGAAGAA	AAGCTAAACC	TCTCAAGCAT	CGCTACCCTA	1860
GACACATTTA	TAGCCCTCAA	TATCTCATTT	ATTGAAAATA	TCGACTTCAA	AACAGTAGAA	1920
CTGTACTCCT	CTACTGAAAG	GAAACTCGCA	TCGAGCGTCT	TTGATATAGA	ATCCATGTTT	1980
AGGGAATATA	ACTATTACAC	CTACAGCCTC	GCGGGCATTA	AGAAGGACCT	AGACAACACC	2040
ATCGACTACA	ATAGAGACAG	ACTGGTTCAG	GACCTGTCTG	ACATGATGGC	TGATCTGGGA	2100
GACATTGGAA	GATCTGTGGT	GAATGTGGTC	AGCTCGGTAG	TCACATTTTT	CAGTAGTATT	2160
GTGACAGGGT	TCATTAAATT	CTTTACCAAC	CCTCTAGGGG	GAATATTCAT	TCTCCTAATT	2220
ATTGGTGGAA	TAATCTTCTT	GGTGGTAGTC	CTAAATAGAA	GAAACTCACA	GTTTCACGAT	2280
GCACCCATCA	AAATGCTGTA	CCCTTCTGTT	GAAAACCTACG	CTGCCAGACA	GGCGCCACCT	2340
CCCTATAGCG	CATCACCTCC	AGCTATAGAC	AAAGAGGAAA	TTAAGCGCAT	ACTTTTGGGC	2400
ATGCATCAGG	TACACCAGGA	AGAAAAGGAA	GCACAGAAAC	AACTAACCAA	CTCTGGCCCT	2460
ACTTTGTGGC	AGAAAGCCAC	AGGATTCCCTT	AGAAATCGCC	GGAAGGGATA	CAGCCAACCTT	2520
CCTCTGGAAG	ATGAATCAAC	TTCCCTCT				2548

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2572 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATGACTCGGC	GTAGGTGCT	AAGCGTGGTC	GTGCTGCTAG	CCGCCCTGGC	GTGCCGTCTC	60
GGTGCGCAGA	CCCCAGAGCA	GCCCGCACCC	CCC GCCACCA	CGGTGCAGCC	TACCGCCACG	120
CGTCAGCAAA	CCAGCTTTCC	TTTCCGAGTC	TGCGAGCTCT	CCAGCCACGG	CGACCTGTTC	180
CGCTTCTCCT	CGGACATCCA	GTGTCCCTCG	TTTGGCACGC	GGGAGAATCA	CACGGAGGGC	240
CTGTTGATGG	TGTTTAAAGA	CAACATTATT	CCCTACTCGT	TTAAGGTCCG	CTCCTACACC	300
AAGATAGTGA	CCAACATTCT	CATCTACAAT	GGCTGGTACG	CGGACTCCGT	GACCAACCGG	360
CACGAGGAGA	AGTTCTCCGT	TGACAGCTAC	GAAACTGACC	AGATGGATAC	CATCTACCAG	420
TGCTACAACG	CGGTCAAGAT	GACAAAAGAT	GGGCTGACGC	GCGTGTATGT	AGACCGCGAC	480
GGAGTTAACA	TCACCGTCAA	CCTAAAGCCC	ACCGGGGGCC	TGGCCAACGG	GGTGCGCCGC	540
TACGCCAGCC	AGACGGAGCT	CTATGACGCC	CCCGGGTGGT	TGATATGGAC	TTACAGAACA	600
AGAACTACCG	TCAACTGCCT	GATAACTGAC	ATGATGGCCA	AGTCCAACAG	CCCTTCGAC	660
TTCTTTGTGA	CCACCACCGG	GCAGACTGTG	GAAATGTCCC	CTTTCTATGA	CGGGAAAAAT	720
AAGGAAACCT	TCCATGAGCG	GGCAGACTCC	TTCCACGTGA	GAAC TAACTA	CAAGATAGTG	780
GACTACGACA	ACCGAGGGAC	GAACCCGCAA	GGCGAACGCC	GAGCCTTCCT	GGACAAGGGC	840
ACTTACACGC	TATCTTGGA	GCTCGAGAAC	AGGACAGCCT	ACTGCCCGCT	TCAACACTGG	900
CAAACCTTTG	ACTCGACCAT	CGCCACAGAA	ACAGGGAAGT	CAATACATTT	TGTGACTGAC	960
GAGGGCACCT	CTAGCTTCGT	GACCAACACA	ACCGTGGGCA	TAGAGCTCCC	GGACGCCTTC	1020
AAGTGCATCG	AAGAGCAGGT	GAACAAGACC	ATGCATGAGA	AGTACGAGGC	CGTCCAGGAT	1080
CGTTACACGA	AGGGCCAGGA	AGCCATTACA	TATTTTATAA	CGAGCGGAGG	ATTGTTATTA	1140
GCTTGCTAC	CTCTGACCCC	GCGCTCGTTG	GCCACCGTCA	AGAACCTGAC	GGAGCTTACC	1200
ACTCCGACTT	CCTCACCCCC	CAGCAGTCCA	TCGCCCCCAG	CCCCATCCGC	GGCCCCGCGG	1260
AGCACCCCCG	CGGCCGTTCT	GAGGCGTCGG	AGGCGGGATG	CGGGGAACGC	CACCACACCG	1320
GTGCCCCCA	CGGCCCCCGG	GAAGTCCCTG	GGCACCCCTCA	ACAATCCCGC	CACCGTCCAG	1380
ATCCAATTTG	CCTACGACTC	CCTGCGCCGC	CAGATCAACC	GCATGCTGGG	AGACCTTGCG	1440
CGGGCCTGGT	GCCTGGAGCA	GAAGAGGCAG	AACATGGTGC	TGAGAGAACT	AACCAAGATT	1500
AATCCAACCA	CCGTCATGTC	CAGCATCTAC	GGTAAGGCGG	TGGCGGCCAA	GCGCCTGGGG	1560
GATGTCATCT	CAGTCTCCCA	GTGCGTGCCC	GTTAACCAGG	CCACCCTCAC	CCTGCGCAAG	1620
AGCATGAGGG	TCCCTGGCTC	CGAGACCATG	TGCTACTCGC	GCCCCCTGGT	GTCCTTCAGC	1680
TTTATCAACG	ACACCAAGAC	CTACGAGGGA	CAGCTGGGCA	CCGACAACGA	GATCTTCTCT	1740
ACAAAAAAGA	TGACGGAGGT	GTGCCAGGCG	ACCAGCCAGT	ACTACTTCCA	GTCCGGCAAC	1800
GAGATCCACG	TCTACAACGA	CTACCACCAC	TTTAAAACCA	TCGAGCTGGA	CGGCATTGCC	1860
ACCCTGCAGA	CCTTCATCTC	ACTAAACACC	TCCCTCATCG	AGAACATTGA	CTTTGCCTCC	1920
CTGGAGCTGT	ACTCACGGGA	CGAACAGCGT	GCCTCCAACG	TCTTTGACCT	GGAGGGCATC	1980
TTCCGGGAGT	ACA ACTTCCA	GGCGAAAAC	ATCGCCGGCC	TGCGGAAGGA	TTTGGACAAT	2040
GCAGTGTCAA	ACGGAAGAAA	TCAATTCGTG	GACGGCCTGG	GGGAACTTAT	GGACAGTCTG	2100
GGTAGCGTGG	GTCAGTCCAT	CACCAACCTA	GTCAGCACGG	TGGGGGGTTT	GTTTAGCAGC	2160
CTGGTCTCTG	GTTTCATCTC	CTTCTTCAAA	AACCCCTTCG	GCGGCATGCT	CATTCTGGTC	2220
CTGGTGGCGG	GGGTGGTGAT	CCTGGTTATT	TCCCTCACGA	GGCGCACGCG	CCAGATGTCG	2280
CAGCAGCCGG	TGCAGATGCT	CTACCCCGGG	ATCGACGAGC	TCGCTCAGCA	ACATGCCTCT	2340

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GGTGAGGGTC CAGGCATTAA TCCCATTAGT AAGACAGAAT TACAAGCCAT CATGTTAGCG	2400
CTGCATGAGC AAAACCAGGA GCAAAAGAGA GCAGCTCAGA GGGCGGCCGG ACCCTCAGTG	2460
GCCAGCAGAG CATTGCAGGC AGCCAGGGAC CGTTTTCAG GCCTACGCAG AAGACGCTAT	2520
CACGATCCAG AGACCGCCGC CGCACTGCTT GGGGAGGCAG AGACTGAGTT TT	2572

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 2722 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ATGGAATCCA GGATCTGGTG CCTGGTAGTC TGCCTTAACC TGTGTATCGT CTGTCTGGGT	60
GCTGCGGTTT CCTCTTCTAG TACTTCCCAT GCAACTTCTT CTACTCACAA TGGAAGCCAT	120
ACTTCTCGTA CGACGTCTGC TCAAACCCGG TCAGTCTATT CTCAACACGT AACGTCTTCT	180
GAAGCCGTC A GTCATAGAGC CAACGAGACT ATCTACAACA CTACCCCTAA GTACGGAGAT	240
GTGGTGGGAG TCAACACTAC CAAGTACCCC TATCGCGTGT GTTCTATGGC CCAGGGTACG	300
GATCTTATTC GCTTTGAACG TAATATCATC TGCACCTCGA TGAAGCCTAT CAATGAAGAC	360
TTGGATGAGG GCATCATGGT GGTCTACAAG CGCAACATCG TGGCGCACAC CTTTAAGGTA	420
CGGGTCTACC AAAAGGTTTT GACGTTTCGT CGTAGCTACG CTTACATCTA CACCACTTAT	480
CTGCTGGGCA GCAATACGGA ATACGTGGCG CCTCCTATGT GGGAGATTCA TCACATCAAC	540
AAGTTTGCTC AATGCTACAG TTCCTACAGC CGCGTTATAG GAGGCACGGT TTTCGTGGCA	600
TATCATAGGG ACAGTTATGA AAACAAAACC ATGCAATTAA TTCCCAGCA TTATTCCAAC	660
ACCCACAGTA CCCGTTACGT GACGGTCAAG GATCAGTGGC ACAGCCGCGG CAGCACCTGG	720
CTCTATCGTG AGACCTGTAA TCTGAACTGT ATGCTGACCA TCACTACTGC GCGCTCCAAG	780
TATCCTTATC ATTTTTTTGC AACTTCCACG GGTGATGTGG TTTACATTTT TCCTTTCTAC	840
AACGGAACCA ATCGCAATGC CAGCTACTTT GGAGAAAACG CCGACAAGTT TTTCAATTTT	900
CCGAACCTACA CCATCGTTTC CGACTTTGGA AGACCCAACG CTGCGCCAGA AACCCATAGG	960
TTGGTGGCTT TTCTCGAACG TGCCGACTCG GTGATCTCTT GGGATATACA GGACGAGAAG	1020
AATGTACACT GCCAGCTCAC CTTCTGGGAA GCCTCGGAAC GTACTATCCG TTCCGAAGCC	1080
GAAGACTCGT ACCACTTTTC TTCTGCCAAA ATGACTGCAA CTTTCTGTGC TAAGAAACAA	1140
GAAGTGAACA TGTCCGACTC CGCGCTGGAC TGGCTACGTG ATGAGGCTAT AAATAAGTTA	1200
CAGCAGATTT TCAATACTTC ATACAATCAA ACATATGAAA AATACGGAAG CGTGTCCGTC	1260
TTCGAAACCA GCGGCGGTCT GGTGGTGTTC TGGCAAGGCA TCAAGCAAAA ATCTTTGGTG	1320
GAATTGGAAC GTTTGGCCAA TCGATCCAGT CTGAATATCA CTCATAGGAC CAGAAGAAGT	1380
ACGAGTGACA ATAATACAAC TCATTTGTCC AGCATGGAAT CGGTGCACAA TCTGGTCTAC	1440
GCCCAGCTGC AGTTCACCTA TGACACGTTG CGCGGTTACA TCAACCGGGC GTGGCGCAA	1500
ATCGCAGAAG CCTGGTGTGT GGATCAACGG CGCACCTTAG AGGTCTTCAA GGAAGTCAGC	1560
AAGATCAACC CGTCAGCCAT TCTCTCGGCC ATTTACAACA AACCGATTGC CGGCGGTTTC	1620
ATGGGTGATG TCTTGGGCTT GGCCAGCTGC GTGACCATCA ACCAAACCAG CGTCAAGGTG	1680
CTGCGTGATA TGAACGTGAA GGAATCGCCA GGACGCTGCT ACTCACGACC CGTGGTCATC	1740
TTTAATTTTC CCAACAGCTC GTACGTGCAG TACGGTCAAC TGGGCGAGGA CAACGAAATC	1800

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CTGTTGGGCA	ACCACCGCAC	TGAGGAATGT	CAGCTTCCCA	GCCTCAAGAT	CTTCATCGCC	1860
GGGAACTCGG	CCTACGAGTA	CGTGGACTAC	CTCTTCAAAC	GCATGATTGA	CCTCAGCAGT	1920
ATCTCCACCG	TCGACAGCAT	GATCGCCCTG	GATATCGACC	CGCTGGAAAA	TACCGACTTC	1980
AGGGTACTGG	AACTTTACTC	GCAGAAAGAG	CTGCGTTCCA	GCAACGTTTT	TGACCTCGAA	2040
GAGATCATGC	GCGAATTCAA	CTCGTACAAG	CAGCGGGTAA	AGTACGTGGA	GGACAAGGTA	2100
GTCGACCCGC	TACCGCCCTA	CCTCAAGGGT	CTGGACGACC	TCATGAGCGG	CCTGGGCGCC	2160
GCGGGAAAGG	CCGTGGCGGT	AGCCATTGGG	GCCGTGGGTG	GCGCGGTGGC	CTCCGTGGTC	2220
GAAGGCGTTG	CCACCTTCCT	CAAAAACCCC	TTCGGAGCCT	TCACCATCAT	CCTCGTGGCC	2280
ATAGCCGTAG	TCATTATCAC	TTATTTGATC	TATACTCGAC	AGCGGCGTCT	GTGCACGCAG	2340
CCGCTGCAGA	ACCTCTTTCC	CTATCTGGTG	TCCGCCGACG	GGACCACCGT	GACGTCGGGC	2400
AGCACCAAAG	ACACGTCGTT	ACAGGCTCCG	CCTTCCTACG	AGGAAAGTGT	TTATAATTCT	2460
GGTCGCAAAG	GACCGGGACC	ACCGTCTGCT	GATGCATCCA	CGGCGGCTCC	GCCTTACACC	2520
AACGAGCAGG	CTTACCAGAT	GCTTCTGGCC	CTGGCCCGTC	TGGACGCAGA	GCAGCGAGCG	2580
CAGCAGAACG	GTACAGATTC	TTTGGACGGA	CAGACTGGCA	CGCAGGACAA	GGGACAGAAG	2640
CCTAACCTGC	TAGACCGGCT	GCGACATCGC	AAAAACGGCT	ACAGACACTT	GAAAGACTCC	2700
GACGAAGAAG	AGAACGTCTG	AA				2722

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 2493 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATGAGCAAGA	TGAGAGTATT	ATTCCTGGCT	GTCTTTTGA	TGAATAGTGT	TTTAATGATA	60
TATTGCGATT	CGGATGATTA	TATCAGAGCG	GGCTATAATC	ACAAATATCC	TTTTCGGATT	120
TGTTTCGATT	CCAAAGGCAC	TGATTTGATG	CGGTCGACA	GAGATATTTT	GTGTTTCGCCA	180
TATAAGTCTA	ATGCAAAGAT	GTCGGAGGGT	TTTTTCATCA	TTTACAAAAC	AAATATCGAG	240
ACCTACACTT	TTCCAGTGAG	AACATATAAA	AACGAGCTGA	CGTTCCAAAC	CAGTTACCGT	300
GATGTGGGTG	TGGTTTATTT	TCTGGATCGG	ACGGTGATGG	GTTTGGCCAT	GCCGGTGTAC	360
GAAGCAAATT	TAGTTAATTC	TCGTGCGCAG	TGTTATTTCAG	CCGTAGCGAT	AAAACGACCC	420
GATGGTACGG	TGTTTAGTGC	CTATCATGAG	GATAATAATA	AAAACGAAAC	TCTAGAATTA	480
TTTCCTCTGA	ATTTCAAGTC	TGTTACTAAT	AAAAGATTTA	TCACTACGAA	AGAACCCTAC	540
TTTGCAAGGG	GTCTTTTGTG	GCTCTATTCT	ACATCGACGT	CTCTCAATTG	TATTGTGACG	600
GAGGCTACGG	CTAAGGCGAA	ATATCCGTTT	AGTTACTTTG	CTTTGACGAC	TGGTGAAATC	660
GTGGAAGGGT	CTCCGTTCTT	CGACGGTTCA	AACGGTAAAC	ATTTTGCAGA	GCCGTTAGAA	720
AAATTGACAA	TCTTGGAATA	CTATACTATG	ATAGAAGATC	TAATGAATGG	TATGAATGGG	780
GCTACTACGT	TAGTAAGGAA	GATCGCTTTT	CTGGAGAAAG	GGGATACTTT	GTTTCTTTGG	840
GAAATCAAGG	AAGAGAATGA	ATCGGTGTGT	ATGCTAAAGC	ACTGGACTAC	GGTGACTCAC	900
GGGCTTCGAG	CGGAGACGGA	TGAGACTTAT	CACCTTTATTT	CTAAGGAGTT	GACAGCCGCT	960
TTCTGTCGCT	CCAAGGAGTC	TTTAAATCTT	ACCGATCCCA	AACAAACGTG	TATTAAGAAT	1020
GAATTTGAGA	AGATAATTAC	AGATGTCTAT	ATGTCAGATT	ATAATGATGA	CTACAGCATG	1080
AACGGTAGTT	ATCAAATTTT	TAAGACTACG	GGAGATCTGA	TTTTGATTGG	GCAGCCTCTT	1140

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GTGCAAAAAT	CTCTTATGGT	TCTTGAGCAG	GGTTCAGTAA	ACTTACGTAG	GAGGCGAGAT	1200
TTGGTGATG	TCAAGTCTAG	ACATGATATT	CTTTATGTGC	AATTACAGTA	CCTCTATGAT	1260
ACTTTGAAAG	ATTATATCAA	CGATGCCTTG	GGGAATTTGG	CAGAATCTTG	GTGCCTCGAT	1320
CAAAAACGAA	CGATAACGAT	GTTGCACGAA	CTTAGTAAGA	TCAGTCCATC	GAGTATCGTG	1380
TC TGAGGTTT	ACGGTCGTCC	GATATCTGCA	CAGTTGCATG	GTGATGTGTT	AGCTATCTCG	1440
AAATGCATAG	AAGTTAATCA	ATCATCCGTT	CAGCTTTATA	AGAGTATGCG	GGTCGTCGAT	1500
GCGAAGGGAG	TAAGGAGTGA	AACGATGTGT	TATAATCGGC	CCTTGGTGAC	GTTTAGCTTT	1560
GTGAAGTCCA	CGCCTGAGGT	TGTCCTTGGT	CAGCTAGGGT	TAGATAATGA	GATTCTGTTG	1620
GGTGATCATA	GGACAGAGGA	ATGTGAGATA	CCTAGTACAA	AGATATTTCT	ATCTGGAAAAT	1680
CATGCACACG	TGTATACCGA	TTATACGCAT	ACGAATTCGA	CGCCCATAGA	AGACATTGAG	1740
GTATTGGATG	CTTTTATTAG	ACTAAAGATC	GACCCCTCTG	AAAATGCTGA	TTTTAAACTA	1800
CTTGATTTAT	ATTCGCCGGA	CGAATTGAGT	AGAGCAAACG	TTTTCGATTT	AGAGAATATT	1860
CTTCGTGAAT	ATAACTCATA	TAAGAGCGCA	CTATATACTA	TAGAAGCTAA	AATTGCTACT	1920
AATACGCCGT	CGTATGTCAA	TGGGATTAAT	TCTTTTTTAC	AAGGGCTTGG	GGCTATAGGC	1980
ACTGGATTGG	GCTCGGTTAT	AAGTGTTACG	GCAGGAGCAC	TTGGGGATAT	TGTGGGTGGA	2040
GTGGTGTCTT	TTTTAAAAAA	TCCATTCGGG	GGTGGTCTCA	TGTTGATTTT	AGCGATAGTA	2100
GTGTGCGTTA	TAATAATTGT	GGTTTTCGTT	AGACAAAAAC	ATGTGCTTAG	TAAGCCTATT	2160
GACATGATGT	TTCCTTATGC	CACCAATCCG	GTGACTACTG	TGTCCAGTGT	TACGGGGACC	2220
ACTGTCGTCA	AGACGCCTAG	TGTTAAAGAT	GCTGACGGGG	GCACATCTGT	TGCGGTTTCG	2280
GAAAAAGAGG	AGGGTATGGC	TGACGTCAGT	GGACAAATAA	GTGGTGATGA	ATATTCACAA	2340
GAAGATGCTT	TAAAAATGCT	CAAGGCCATA	AAGTCTTTAG	ACGAGTCCTA	CAGAAGAAAA	2400
CCTTCGTCTT	CTGAGTCTCA	TGCCTCAAAA	CCTAGTTTGA	TAGACAGGAT	CAGGTATAGA	2460
GGTTATAAGA	GTGTAAATGT	AGAAGAAGCG	TGA			2493

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 2608 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ATGTTTGTTA	CGGCGGTTGT	GTCGGTCTCT	CCAAGCTCGT	TTTATGAGAG	TTTACAAGTA	60
GAGCCCACAC	AATCAGAAGA	TATAACCCGG	TCTGCTCATC	TGGGCGATGG	TGATGAAATC	120
AGAGAAGCTA	TACACAAGTC	CCAGGACGCC	GAAACAAAAC	CCACGTTTTA	CGTCTGCCCA	180
CCGCCAACAG	GCTCCACAAT	CGTACGATTA	GAACCAACTC	GGACATGTCC	GGATTATCAC	240
CTTGGTAAAA	ACTTTACAGA	GGGTATTGCT	GTTGTTTATA	AAGAAAACAT	TGCAGCGTAC	300
AAGTTTAAGG	CGACGGTATA	TTACAAAGAT	GTTATCGTTA	GCACGGCGTG	GGCCGGAAGT	360
TCTTATACGC	AAATTAATAA	TAGATATGCG	GATAGGGTAC	CAATTCCCGT	TTCAGAGATC	420
ACGGACACCA	TTGATAAGTT	TGGCAAGTGT	TCTTCTAAAG	CAACGTACGT	ACGAAATAAC	480
CACAAAGTTG	AAGCCTTTAA	TGAGGATAAA	AATCCACAGG	ATATGCCTCT	AATCGCATCA	540
AAATATAATT	CTGTGGGATC	CAAAGCATGG	CATACTACCA	ATGACACGTA	CATGGTTGCC	600
GGAACCCCGG	GAACATATAG	GACGGGCACG	TCGGTGAATT	GCATCATTGA	GGAAGTTGAA	660

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GCCAGATCAA	TATTCCCTTA	TGATAGTTTT	GGACTTTCCA	CGGGAGATAT	AATATACATG	720
TCCCCGTTTT	TTGGCCTACG	GGATGGTGCA	TACAGAGAAC	ATTCCAATTA	TGCAATGGAT	780
CGTTTTCACC	AGTTTGAGGG	TTATAGACAA	AGGGATCTTG	ACACTAGAGC	ATTACTGGAA	840
CCTGCAGCGC	GGAACCTTTT	AGTCACGCCT	CATTTAACGG	TTGGTTGGAA	CTGGAAGCCA	900
AAACGAACGG	AAGTTTGTTC	GCTTGTCAAG	TGGCGTGAGG	TTGAAGACGT	AGTTCGCGAT	960
GAGTATGCAC	ACAATTTTCG	CTTTACAATG	AAAACACTTT	CTACCACGTT	TATAAGTGAA	1020
ACAAACGAGT	TTAATCTTAA	CCAAATCCAT	CTCAGTCAAT	GTGTAAAGGA	GGAAGCCCGG	1080
GCTATTATTA	ACCGGATCTA	TACAACCAGA	TACAACTCAT	CTCATGTTAG	AACCGGGGAT	1140
ATCCAGACCT	ACCTTGCCAG	AGGGGGGTTT	GTTGTGGTGT	TTCAACCCCT	GCTGAGCAAT	1200
TCCCTCGCCC	GTCTCTATCT	CCAAGAATTG	GTCCGTGAAA	ACACTAATCA	TTCACCACAA	1260
AAACACCCGA	CTCGAAATAC	CAGATCCCGA	CGAAGCGTGC	CAGTTGAGTT	GCGTGCCAAT	1320
AGAACAATAA	CAACCACCTC	ATCGGTGGAA	TTTGCTATGC	TCCAGTTTAC	ATATGACCAC	1380
ATTCAAGAGC	ATGTTAATGA	AATGTTGGCA	CGTATCTCCT	CGTCGTGGTG	CCAGCTACAA	1440
AATCGCGAAC	GCGCCCTTTG	GAGCGGACTA	TTTCCAATTA	ACCCAAGTGC	TTTAGCGAGC	1500
ACCATTTTGG	ATCAACGTGT	TAAAGCTCGT	ATTCTCGGCG	ACGTTATCTC	CGTTTCTAAT	1560
TGTCCAGAAC	TGGGATCAGA	TACACGCATT	ATACTTCAAA	ACTCTATGAG	GGTATCTGGT	1620
AGTACTACGC	GTTGTTATAG	CCGTCCTTTA	ATTTCAATAG	TTAGTTTAAA	TGGGTCCGGG	1680
ACGGTGGAGG	GCCAGCTTGG	AACAGATAAC	GAGTTAATTA	TGTCCAGAGA	TCTGTTAGAA	1740
CCATGCGTGG	CTAATCACAA	GCGATATTTT	CTATTTGGGC	ATCACTACGT	ATATTATGAG	1800
GATTATCGTT	ACGTCCGTGA	AATCGCAGTC	CATGATGTGG	GAATGATTAG	CACTTACGTA	1860
GATTTAAACT	TAACACTTCT	TAAAGATAGA	GAGTTTATGC	CGCTGCAAGT	ATATACAAGA	1920
GACGAGCTGC	GGGATACAGG	ATTACTAGAC	TACAGTGAAA	TTCAACGCCG	AAATCAAATG	1980
CATTCGCTGC	GTTTTTATGA	CATAGACAAG	GTTGTGCAAT	ATGATAGCGG	AACGGCCATT	2040
ATGCAGGGCA	TGGCTCAGTT	TTTCCAGGGA	CTTGGGACCG	CGGGCCAGGC	CGTTGGACAT	2100
GTGGTTCTTG	GGGCCACGGG	AGCGCTGCTT	TCCACCGTAC	ACGGATTTAC	CACGTTTTTA	2160
TCTAACCCAT	TTGGGGCATT	GGCCGTGGGA	TTATTGGTTT	TGGCGGGACT	GGTAGCGGCC	2220
TTTTTTGCGT	ACCGGTACGT	GCTTAAACTT	AAAACAAGCC	CGATGAAGGC	ATTATATCCA	2280
CTCACAACCA	AGGGGTTAAA	ACAGTTACCG	GAAGGAATGG	ATCCCTTTGC	CGAGAAACCC	2340
AACGCTACTG	ATACCCCAAT	AGAAGAAATT	GGCGACTCAC	AAAACACTGA	ACCGTCGGTA	2400
AATAGCGGGT	TTGATCCCGA	TAAATTTCGA	GAAGCCCAGG	AAATGATTAA	ATATATGACG	2460
TTAGTATCTG	CGGTGAGCG	CCAAGAATCT	AAAGCCCACA	AAAAAATAA	GACTAGCGCC	2520
CTTTTAACTT	CACGTCTTAC	CGGCCTTGCT	TTACGAAATC	GCCGAGGATA	CTCCCGTGTT	2580
CGCACCGAGA	ATGTAACGGG	GGTGTAAT				2608

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2713 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATGCGCCAGG	GCGCCGCGCG	GGGGTGCCGG	TGGTTCGTCTG	TATGGGCGCT	CTTGGGGTTG	60
ACGCTGGGGG	TCCTGGTGCG	GTCGGCGGCT	CCGAGTTCCC	CCGGCACGCC	TGGGGTCGCG	120

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GCCGCGACCC	AGGCGGCGAA	CGGGGGACCT	GCCACTCCGG	CGCCGCCCGC	CCCTGGCCCC	180
GCCCCAACGG	GGGACACGAA	ACCGAAGAAG	AACAAAAAAC	CGAAAAACCC	ACCGCCGCCG	240
CGCCCCGCCG	GCGACAACGC	GACCGTCGCC	GCGGGCCACG	CCACCCTGCG	CGAGCACCTG	300
CGGGACATCA	AGGCGGAGAA	CACCGATGCA	AACTTTTACG	TGTGCCCACC	CCCCACGGGC	360
GCCACGGTGG	TGCAGTTTCA	GCAAGCCGCG	CGCTGCCCGA	CCCGGCCCGA	GGGTGAGAAC	420
TACACGGAGG	GCATCGCGGT	GGTCTTCAAG	GAGAACATCG	CCCCGTACAA	GTTCAAGGCC	480
ACCATGTACT	ACAAAGACGT	CACCGTTTCG	CAGGTGTGGT	TCGGCCACCG	CTACTCCCAG	540
TTTATGGGGA	TCTTTGAGGA	CCGCGCCCCC	GTCCCCTTCG	AGGAGGTGAT	CGACAAGATC	600
AACGCCAAGG	GGGTCTGTCT	GTCCACGGCC	AAGTACGTGC	GCAACAACCT	GGAGACCACC	660
GCGTTTCACC	GGGACGACCA	CGAGACCGAC	ATGGAGCTGA	AACCGGCCAA	CGCCGCGACC	720
CGCACGAGCC	GGGGCTGGCA	CACCACCGAC	CTCAAGTACA	ACCCCTCGCG	GGTGGAGGCG	780
TTCCACCGGT	ACGGGACGAC	GGTAAACTGC	ATCGTCGAGG	AGGTGGACGC	GCGCTCGGTG	840
TACCCGTACG	ACGAGTTTGT	GCTGGCGACT	GGCGACTTTG	TGTACATGTC	CCCGTTTTC	900
GGCTACCGGG	AGGGGTCGCA	CACCGAACAC	ACCAGCTACG	CCGCCGACCG	CTTCAAGCAG	960
GTTCGACGGT	TCTACGCGCG	CGACCTCACC	ACCAAGGCCC	GGGCCACGGC	GCCGACCACC	1020
CGGAACCTGC	TCACGACCCC	CAAGTTCACC	GTGGCCTGGG	ACTGGGTGCC	AAAGCGCCCC	1080
TCGGTCTGCA	CCATGACCAA	GTGGCAGGAG	GTGGACGAGA	TGCTGCGCTC	CGAGTACGGC	1140
GGCTCCTTCC	GATTCTCCTC	CGACGCCATA	TCCACCACCT	TCACCACCAA	CCTGACCGAG	1200
TACCCGTCTC	CGCGCGTGGA	CCTGGGGGAC	TGCATCGGCA	AGGACGCCCG	CGACGCCATG	1260
GACCGCATCT	TCGCCCCGAG	GTACAACGCG	ACGCACATCA	AGGTGGGCCA	GCCGCAGTAC	1320
TACCTGGCCA	ATGGGGGCTT	TCTGATCGCG	TACCAGCCCC	TTCTCAGCAA	CACGCTCGCG	1380
GAGCTGTACG	TGCGGGAACA	CCTCCGAGAG	CAGAGCCGCA	AGCCCCCAAA	CCCCACGCCC	1440
CCGCCGCCCC	GGGCCAGCGC	CAACGCGTCC	GTGGAGCGCA	TCAAGACCAC	CTCCTCCATC	1500
GAGTTCGCCC	GGCTGCAGTT	TACGTACAAC	CACATACAGC	GCCATGTCAA	CGATATGTTG	1560
GGCCGCGTTG	CCATCGCGTG	GTGCGAGCTG	CAGAATCACG	AGCTGACCCT	GTGGAACGAG	1620
GCCCCGAAGC	TGAACCCCAA	GCCTATCGCC	TCGGCCACCG	TGGGCCGGCG	GGTGAGCGCG	1680
CGGATGCTCG	GCGACGTGAT	GGCCGTCTCC	ACGTGCGTGC	CGGTGCGCGC	GGACAACGTG	1740
ATCGTCCAAA	ACTCGATGCG	CATCAGCTCG	CGGCCCGGGG	CCTGCTACAG	CCGCCCCCTG	1800
GTCAGCTTTC	GGTACGAAGA	CCAGGGCCCC	TTGGTCGAGG	GGCAGGTGGG	GGAGAACAAC	1860
GAGCTGCGGC	TGACGCGCGA	TGCGATCGAG	CCGTGCACCG	TGGGACACCG	GCGCTACTTC	1920
ACCTTCGGTG	GGGGCTACGT	GTAATTTCGAG	GAGTACGCGT	ACTCCCACCA	GCTGAGCCGC	1980
GCCGACATCA	CCACCGTCAG	CACCTTCATC	GACCTCAACA	TCACCATGCT	GGAGGATCAC	2040
GAGTTTGTCC	CCCTGGAGGT	GTACACCCGC	CACGAGATCA	AGGACAGCGG	CCTGCTGGAC	2100
TACACGGAGG	TCCAGCGCCG	CAACCAGCTG	CACGACCTGC	GCTTCGCCGA	CATCGACACG	2160
GTCATCCACG	CCGACGCCAA	CGCCGCCATG	TTGCGGGGCC	TGGGCGCGTT	CTTCGAGGGG	2220
ATGGGCGACC	TGGGGCGCGC	GGTCGGCAAG	GTGGTGATGG	GCATCGTGGG	CGGCGTGGTA	2280
TCGGCCGTGT	CGGGCGTGTC	CTCCTTCATG	TCCAACCCCT	TTGGGGCGCT	GGCCGTGGGT	2340
CTGTTGGTCC	TGGCCGGCCT	GGCGGCGGCT	TTCTTCGCCT	TTGCTACGTC	CATGCGGCTG	2400
CAGAGCAACC	CCATGAAGGC	CCTGTACCCG	CTAACCACCA	AGGAGCTCAA	GAACCCACCC	2460
AACCCGGACG	CGTCCGGGGA	GGGCGAGGAG	GGCGGCGACT	TTGACGAGGC	CAAGCTAGCC	2520

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GAGGCCCGGG AGATGATACG GTACATGGCC CTGGTGTCTG CCATGGAGCG CACGGAACAC 2580
 AAGGCCAAGA AGAAGGGCAC GAGCGCGCTG CTCAGCGCCA AGGTCACCGA CATGGTCATG 2640
 CGCAAGCGCC GCAACACCAA CTACACCCAA GTTCCCAACA AAGACGGTGA CGCCGACGAG 2700
 GACGACCTGT GAC 2713

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 808 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Val Pro Asn Lys His Leu Leu Leu Ile Ile Leu Ser Phe Ser Thr
 1 5 10 15
 Ala Cys Gly Gln Thr Thr Pro Thr Thr Ala Val Glu Lys Asn Lys Thr
 20 25 30
 Gln Ala Ile Tyr Gln Glu Tyr Phe Lys Tyr Arg Val Cys Ser Ala Ser
 35 40 45
 Thr Thr Gly Glu Leu Phe Arg Phe Asp Leu Asp Arg Thr Cys Pro Ser
 50 55 60
 Thr Glu Asp Lys Val His Lys Glu Gly Ile Leu Leu Val Tyr Lys Lys
 65 70 75 80
 Asn Ile Val Pro Tyr Ile Phe Lys Val Arg Arg Tyr Lys Lys Ile Thr
 85 90 95
 Thr Ser Val Arg Ile Phe Asn Gly Trp Thr Arg Glu Gly Val Ala Ile
 100 105 110
 Thr Asn Lys Trp Glu Leu Ser Arg Ala Val Pro Lys Tyr Glu Ile Asp
 115 120 125
 Ile Met Asp Lys Thr Tyr Gln Cys His Asn Cys Met Gln Ile Glu Val
 130 135 140
 Asn Gly Met Leu Asn Ser Tyr Tyr Asp Arg Asp Gly Asn Asn Lys Thr
 145 150 155 160
 Val Asp Leu Lys Pro Val Asp Gly Leu Thr Gly Ala Ile Thr Arg Tyr
 165 170 175
 Ile Ser Gln Pro Lys Val Phe Ala Asp Pro Gly Trp Leu Trp Gly Thr
 180 185 190
 Tyr Arg Thr Arg Thr Thr Val Asn Cys Glu Ile Val Asp Met Phe Ala
 195 200 205
 Arg Ser Ala Asp Pro Tyr Thr Tyr Phe Val Thr Ala Leu Gly Asp Thr
 210 215 220
 Val Glu Val Ser Pro Phe Cys Asp Val Asp Asn Ser Cys Pro Asn Ala
 225 230 235 240
 Thr Asp Val Leu Ser Val Gln Ile Asp Leu Asn His Thr Val Val Asp
 245 250 255
 Tyr Gly Asn Arg Ala Thr Ser Gln Gln His Lys Lys Arg Ile Phe Ala
 260 265 270
 His Thr Leu Asp Tyr Ser Val Ser Trp Glu Ala Val Asn Lys Ser Ala
 275 280 285
 Ser Val Cys Ser Met Val Phe Trp Lys Ser Phe Gln Arg Ala Ile Gln
 290 295 300
 Thr Glu His Asp Leu Thr Tyr His Phe Ile Ala Asn Glu Ile Thr Ala

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305	310	315	320
Gly Phe Ser Thr Val Lys Glu Pro Leu Ala Asn Phe Thr Ser Asp Tyr	325	330	335
Asn Cys Leu Met Thr His Ile Asn Thr Thr Leu Glu Asp Lys Ile Ala	340	345	350
Arg Val Asn Asn Thr His Thr Pro Asn Gly Thr Ala Glu Tyr Tyr Gln	355	360	365
Thr Glu Gly Gly Met Ile Leu Val Trp Gln Pro Leu Ile Ala Ile Glu	370	375	380
Leu Glu Glu Ala Met Leu Glu Ala Thr Thr Ser Pro Val Thr Pro Ser	385	390	395
Ala Pro Thr Ser Ser Ser Arg Ser Lys Arg Ala Ile Arg Ser Ile Arg	405	410	415
Asp Val Ser Ala Gly Ser Glu Asn Asn Val Phe Leu Ser Gln Ile Gln	420	425	430
Tyr Ala Tyr Asp Lys Leu Arg Gln Ser Ile Asn Asn Val Leu Glu Glu	435	440	445
Leu Ala Ile Thr Trp Cys Arg Glu Gln Val Arg Gln Thr Met Val Trp	450	455	460
Tyr Glu Ile Ala Lys Ile Asn Pro Thr Ser Val Met Thr Ala Ile Tyr	465	470	475
Gly Lys Pro Val Ser Arg Lys Ala Leu Gly Asp Val Ile Ser Val Thr	485	490	495
Glu Cys Ile Asn Val Asp Gln Ser Ser Val Ser Ile His Lys Ser Leu	500	505	510
Lys Thr Glu Asn Asn Asp Ile Cys Tyr Ser Arg Pro Pro Val Thr Phe	515	520	525
Lys Phe Val Asn Ser Ser Gln Leu Phe Lys Gly Gln Leu Gly Ala Arg	530	535	540
Asn Glu Ile Leu Leu Ser Glu Ser Leu Val Glu Asn Cys His Gln Asn	545	550	555
Ala Glu Thr Phe Phe Thr Ala Lys Asn Glu Thr Tyr His Phe Lys Asn	565	570	575
Tyr Val His Val Glu Thr Leu Pro Val Asn Asn Ile Ser Thr Leu Asp	580	585	590
Thr Phe Leu Ala Leu Asn Leu Thr Phe Ile Glu Asn Ile Asp Phe Lys	595	600	605
Ala Val Glu Leu Tyr Ser Ser Gly Glu Arg Lys Leu Ala Asn Val Phe	610	615	620
Asp Leu Glu Thr Met Phe Arg Glu Tyr Asn Tyr Tyr Ala Gln Ser Ile	625	630	635
Ser Gly Leu Arg Lys Asp Phe Asp Asn Ser Gln Arg Asn Asn Arg Asp	645	650	655
Arg Ile Ile Gln Asp Phe Ser Glu Ile Leu Ala Asp Leu Gly Ser Ile	660	665	670
Gly Lys Val Ile Val Asn Val Ala Ser Gly Ala Phe Ser Leu Phe Gly	675	680	685
Gly Ile Val Thr Gly Ile Leu Asn Phe Ile Lys Asn Pro Leu Gly Gly	690	695	700
Met Phe Thr Phe Leu Leu Ile Gly Ala Val Ile Ile Leu Val Ile Leu	705	710	715
Leu Val Arg Arg Thr Asn Asn Met Ser Gln Ala Pro Ile Arg Met Ile	725	730	735

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Tyr Pro Asp Val Glu Lys Ser Lys Ser Thr Val Thr Pro Met Glu Pro
740 745 750

Glu Thr Ile Lys Gln Ile Leu Leu Gly Met His Asn Met Gln Gln Glu
755 760 765

Ala Tyr Lys Lys Lys Glu Glu Gln Arg Ala Ala Arg Pro Ser Ile Phe
770 775 780

Arg Gln Ala Ala Glu Thr Phe Leu Arg Lys Arg Ser Gly Tyr Lys Gln
785 790 795 800

Ile Ser Thr Glu Asp Lys Ile Val
805

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 874 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Met Tyr Tyr Lys Thr Ile Leu Phe Phe Ala Leu Ile Lys Val Cys Ser
1 5 10 15

Phe Asn Gln Thr Thr Thr His Ser Thr Thr Thr Ser Pro Ser Ile Ser
20 25 30

Ser Thr Thr Ser Ser Thr Thr Thr Ser Thr Ser Lys Pro Ser Asn Thr
35 40 45

Thr Ser Thr Asn Ser Ser Leu Ala Ala Ser Pro Gln Asn Thr Ser Thr
50 55 60

Ser Lys Pro Ser Thr Asp Asn Gln Gly Thr Ser Thr Pro Thr Ile Pro
65 70 75 80

Thr Val Thr Asp Asp Thr Ala Ser Lys Asn Phe Tyr Lys Tyr Arg Val
85 90 95

Cys Ser Ala Ser Ser Ser Ser Gly Glu Leu Phe Arg Phe Asp Leu Asp
100 105 110

Gln Thr Cys Pro Asp Thr Lys Asp Lys Lys His Val Glu Gly Ile Leu
115 120 125

Leu Val Leu Lys Lys Asn Ile Val Pro Tyr Ile Phe Lys Val Arg Lys
130 135 140

Tyr Arg Lys Ile Ala Thr Ser Val Thr Val Tyr Arg Gly Trp Ser Gln
145 150 155 160

Ala Ala Val Thr Asn Arg Asp Asp Ile Ser Arg Ala Ile Pro Tyr Asn
165 170 175

Glu Ile Ser Met Ile Asp Arg Thr Tyr His Cys Phe Ser Ala Met Ala
180 185 190

Thr Val Ile Asn Gly Ile Leu Asn Thr Tyr Ile Asp Arg Asp Ser Glu
195 200 205

Asn Lys Ser Val Pro Leu Gln Pro Val Ala Gly Leu Thr Glu Asn Ile
210 215 220

Asn Arg Tyr Phe Ser Gln Pro Leu Ile Tyr Ala Glu Pro Gly Trp Phe
225 230 235 240

Pro Gly Ile Tyr Arg Val Arg Thr Thr Val Asn Cys Glu Val Val Asp
245 250 255

Met Tyr Ala Arg Ser Val Glu Pro Tyr Thr His Phe Ile Thr Ala Leu
260 265 270

Gly Asp Thr Ile Glu Ile Ser Pro Phe Cys His Asn Asn Ser Gln Cys

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275					280					285					
Thr	Thr	Gly	Asn	Ser	Thr	Ser	Arg	Asp	Ala	Thr	Lys	Val	Trp	Ile	Glu
	290					295					300				
Glu	Asn	His	Gln	Thr	Val	Asp	Tyr	Glu	Arg	Arg	Gly	His	Pro	Thr	Lys
305					310					315					320
Asp	Lys	Arg	Ile	Phe	Leu	Lys	Asp	Glu	Glu	Tyr	Thr	Ile	Ser	Trp	Lys
				325					330					335	
Ala	Glu	Asp	Arg	Glu	Arg	Ala	Ile	Cys	Asp	Phe	Val	Ile	Trp	Lys	Thr
				340				345					350		
Phe	Pro	Arg	Ala	Ile	Gln	Thr	Ile	His	Asn	Glu	Ser	Phe	His	Phe	Val
		355					360					365			
Ala	Asn	Glu	Val	Thr	Ala	Ser	Phe	Leu	Thr	Ser	Asn	Gln	Glu	Glu	Thr
	370					375					380				
Glu	Leu	Arg	Gly	Asn	Thr	Glu	Ile	Leu	Asn	Cys	Met	Asn	Ser	Thr	Ile
385					390					395					400
Asn	Glu	Thr	Leu	Glu	Glu	Thr	Val	Lys	Lys	Phe	Asn	Lys	Ser	His	Ile
				405					410					415	
Arg	Asp	Gly	Glu	Val	Lys	Tyr	Tyr	Lys	Thr	Asn	Gly	Gly	Leu	Phe	Leu
			420					425					430		
Ile	Trp	Gln	Ala	Met	Lys	Pro	Leu	Asn	Leu	Ser	Glu	His	Thr	Asn	Tyr
	435						440					445			
Thr	Ile	Glu	Arg	Asn	Asn	Lys	Thr	Gly	Asn	Lys	Ser	Arg	Gln	Lys	Arg
	450					455					460				
Ser	Val	Asp	Thr	Lys	Thr	Phe	Gln	Gly	Ala	Lys	Gly	Leu	Ser	Thr	Ala
465					470					475					480
Gln	Val	Gln	Tyr	Ala	Tyr	Asp	His	Leu	Arg	Thr	Ser	Met	Asn	His	Ile
				485					490					495	
Leu	Glu	Glu	Leu	Thr	Lys	Thr	Trp	Cys	Arg	Glu	Gln	Lys	Lys	Asp	Asn
			500					505					510		
Leu	Met	Trp	Tyr	Glu	Leu	Ser	Lys	Ile	Asn	Pro	Val	Ser	Val	Met	Ala
	515						520					525			
Ala	Ile	Tyr	Gly	Lys	Pro	Val	Ala	Val	Lys	Ala	Met	Gly	Asp	Ala	Phe
	530					535					540				
Met	Val	Ser	Glu	Cys	Ile	Asn	Val	Asp	Gln	Ala	Ser	Val	Asn	Ile	His
545					550					555					560
Lys	Ser	Met	Arg	Thr	Asp	Asp	Pro	Lys	Val	Cys	Tyr	Ser	Arg	Pro	Leu
				565					570					575	
Val	Thr	Phe	Lys	Phe	Val	Asn	Ser	Thr	Ala	Thr	Phe	Arg	Gly	Gln	Leu
			580					585					590		
Gly	Thr	Arg	Asn	Glu	Ile	Leu	Leu	Thr	Asn	Thr	His	Val	Glu	Thr	Cys
	595						600					605			
Arg	Pro	Thr	Ala	Asp	His	Tyr	Phe	Phe	Val	Lys	Asn	Met	Thr	His	Tyr
	610					615					620				
Phe	Lys	Asp	Tyr	Lys	Phe	Val	Lys	Thr	Met	Asp	Thr	Asn	Asn	Ile	Ser
625					630					635					640
Thr	Leu	Asp	Thr	Phe	Leu	Thr	Leu	Asn	Leu	Thr	Phe	Ile	Asp	Asn	Ile
				645					650					655	
Asp	Phe	Lys	Thr	Val	Glu	Leu	Tyr	Ser	Glu	Thr	Glu	Arg	Lys	Met	Ala
			660					665					670		
Ser	Ala	Leu	Asp	Leu	Glu	Thr	Met	Phe	Arg	Glu	Tyr	Asn	Tyr	Tyr	Thr
	675						680					685			
Gln	Lys	Leu	Ala	Ser	Leu	Arg	Glu	Asp	Leu	Asp	Asn	Thr	Ile	Asp	Leu
	690					695					700				

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180						185						190					
Pro	Glu	Val	Tyr	Ala	Thr	Pro	Arg	Asn	Leu	Leu	Trp	Ser	Tyr	Thr	Thr		
		195					200					205					
Arg	Thr	Thr	Val	Asn	Cys	Glu	Val	Thr	Glu	Met	Ser	Ala	Arg	Ser	Met		
		210				215					220						
Lys	Pro	Phe	Glu	Phe	Phe	Val	Thr	Ser	Val	Gly	Asp	Thr	Ile	Glu	Met		
225					230					235					240		
Ser	Pro	Phe	Leu	Lys	Glu	Asn	Gly	Thr	Glu	Pro	Glu	Lys	Ile	Leu	Lys		
				245					250					255			
Arg	Pro	His	Ser	Ile	Gln	Leu	Leu	Lys	Asn	Tyr	Ala	Val	Thr	Lys	Tyr		
			260					265					270				
Gly	Val	Gly	Leu	Gly	Gln	Ala	Asp	Asn	Ala	Thr	Arg	Phe	Phe	Ala	Ile		
		275					280					285					
Phe	Gly	Asp	Tyr	Ser	Leu	Ser	Trp	Lys	Ala	Thr	Thr	Glu	Asn	Ser	Ser		
	290					295					300						
Tyr	Cys	Asp	Leu	Ile	Leu	Trp	Lys	Gly	Phe	Ser	Asn	Ala	Ile	Gln	Thr		
305					310					315					320		
Gln	His	Asn	Ser	Ser	Leu	His	Phe	Ile	Ala	Asn	Asp	Ile	Thr	Ala	Ser		
				325					330					335			
Phe	Ser	Thr	Pro	Leu	Glu	Glu	Glu	Ala	Asn	Phe	Asn	Glu	Thr	Phe	Lys		
			340					345					350				
Cys	Ile	Trp	Asn	Asn	Thr	Gln	Glu	Glu	Ile	Gln	Lys	Lys	Leu	Lys	Glu		
		355					360					365					
Val	Glu	Lys	Thr	His	Arg	Pro	Asn	Gly	Thr	Ala	Lys	Val	Tyr	Lys	Thr		
	370					375					380						
Thr	Gly	Asn	Leu	Tyr	Ile	Val	Trp	Gln	Pro	Leu	Ile	Gln	Ile	Asp	Leu		
385					390					395					400		
Leu	Asp	Thr	His	Ala	Lys	Leu	Tyr	Asn	Leu	Thr	Asn	Ala	Thr	Ala	Ser		
				405					410					415			
Pro	Thr	Ser	Thr	Pro	Thr	Thr	Ser	Pro	Arg	Arg	Arg	Arg	Arg	Asp	Thr		
			420					425					430				
Ser	Ser	Val	Ser	Gly	Gly	Gly	Asn	Asn	Gly	Asp	Asn	Ser	Thr	Lys	Glu		
		435					440					445					
Glu	Ser	Val	Ala	Ala	Ser	Gln	Val	Gln	Phe	Ala	Tyr	Asp	Asn	Leu	Arg		
	450					455					460						
Lys	Ser	Ile	Asn	Arg	Val	Leu	Gly	Glu	Leu	Ser	Arg	Ala	Trp	Cys	Arg		
465					470					475					480		
Glu	Gln	Tyr	Arg	Ala	Ser	Leu	Met	Trp	Tyr	Glu	Leu	Ser	Lys	Ile	Asn		
				485					490					495			
Pro	Thr	Ser	Val	Met	Ser	Ala	Ile	Tyr	Gly	Arg	Pro	Val	Ser	Ala	Lys		
			500					505					510				
Leu	Ile	Gly	Asp	Val	Val	Ser	Val	Ser	Asp	Cys	Ile	Ser	Val	Asp	Gln		
		515					520					525					
Lys	Ser	Val	Phe	Val	His	Lys	Asn	Met	Lys	Val	Pro	Gly	Lys	Glu	Asp		
	530					535					540						
Leu	Cys	Tyr	Thr	Arg	Pro	Val	Val	Gly	Phe	Lys	Phe	Ile	Asn	Gly	Ser		
545					550				555						560		
Glu	Leu	Phe	Ala	Gly	Gln	Leu	Gly	Pro	Arg	Asn	Glu	Ile	Val	Leu	Ser		
				565					570					575			
Thr	Ser	Gln	Val	Glu	Val	Cys	Gln	His	Ser	Cys	Glu	His	Tyr	Phe	Gln		
			580					585					590				
Ala	Gly	Asn	Gln	Met	Tyr	Lys	Tyr	Lys	Asp	Tyr	Tyr	Tyr	Val	Ser	Thr		
	595						600					605					

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Leu Asn Leu Thr Asp Ile Pro Thr Leu His Thr Met Ile Thr Leu Asn
 610                               615                               620

Leu Ser Leu Val Glu Asn Ile Asp Phe Lys Val Ile Glu Leu Tyr Ser
625                               630                               635                               640

Lys Thr Glu Lys Arg Leu Ser Asn Val Phe Asp Ile Glu Thr Met Phe
                               645                               650                               655

Arg Glu Tyr Asn Tyr Tyr Thr Gln Asn Leu Asn Gly Leu Arg Lys Asp
                               660                               665                               670

Leu Asp Asp Ser Ile Asp His Gly Arg Asp Ser Phe Ile Gln Thr Leu
675                               680                               685

Gly Asp Ile Met Gln Asp Leu Gly Thr Ile Gly Lys Val Val Val Asn
690                               695                               700

Val Ala Ser Gly Val Phe Ser Leu Phe Gly Ser Ile Val Ser Gly Val
705                               710                               715                               720

Ile Ser Phe Phe Lys Asn Pro Phe Gly Gly Met Leu Leu Ile Val Leu
                               725                               730                               735

Ile Ile Ala Gly Val Val Val Val Tyr Leu Phe Met Thr Arg Ser Arg
                               740                               745                               750

Ser Ile Tyr Ser Ala Pro Ile Arg Met Leu Tyr Pro Gly Val Glu Arg
755                               760                               765

Ala Ala Gln Glu Pro Gly Ala His Pro Val Ser Glu Asp Gln Ile Arg
770                               775                               780

Asn Ile Leu Met Gly Met His Gln Phe Gln Gln Arg Gln Arg Ala Glu
785                               790                               795                               800

Glu Glu Ala Arg Arg Glu Glu Glu Val Lys Gly Lys Arg Thr Leu Phe
                               805                               810                               815

Glu Val Ile Arg Asp Ser Ala Thr Ser Val Leu Arg Arg Arg Arg Gly
                               820                               825                               830

Gly Gly Gly Tyr Gln Arg Leu Gln Arg Asp Gly Ser Asp Asp Glu Gly
835                               840                               845

Asp Tyr Glu Pro Leu Arg Arg Gln Asp Gly Gly Tyr Asp Asp Val Asp
850                               855                               860

Val Glu Ala Gly Thr Ala Asp Thr Gly Val
865                               870

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(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 849 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

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Met Tyr Pro Thr Val Lys Ser Met Arg Val Ala His Leu Thr Asn Leu
 1                               5                               10                               15

Leu Thr Leu Leu Cys Leu Leu Cys His Thr His Leu Tyr Val Cys Gln
                               20                               25                               30

Pro Thr Thr Leu Arg Gln Pro Ser Asp Met Thr Pro Ala Gln Asp Ala
                               35                               40                               45

Pro Thr Glu Thr Pro Pro Pro Leu Ser Thr Asn Thr Asn Arg Gly Phe
50                               55                               60

Glu Tyr Phe Arg Val Cys Gly Val Ala Ala Thr Gly Glu Thr Phe Arg
65                               70                               75                               80

Phe Asp Leu Asp Lys Thr Cys Pro Ser Thr Gln Asp Lys Lys His Val

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85								90					95				
Glu	Gly	Ile	Leu 100	Leu	Val	Tyr	Lys	Ile 105	Asn	Ile	Val	Pro	Tyr 110	Ile	Phe		
Lys	Ile	Arg 115	Arg	Tyr	Arg	Lys	Ile 120	Ile	Thr	Gln	Leu	Thr 125	Ile	Trp	Arg		
Gly	Leu 130	Thr	Thr	Ser	Ser	Val 135	Thr	Gly	Lys	Phe	Glu 140	Met	Ala	Thr	Gln		
Ala 145	His	Glu	Trp	Glu	Val 150	Gly	Asp	Phe	Asp	Ser 155	Ile	Tyr	Gln	Cys	Tyr 160		
Asn	Ser	Ala	Thr	Met 165	Val	Val	Asn	Asn	Val 170	Arg	Gln	Val	Tyr	Val 175	Asp		
Arg	Asp	Gly	Val 180	Asn	Lys	Thr	Val	Asn 185	Ile	Arg	Pro	Val	Asp 190	Gly	Leu		
Thr	Gly	Asn 195	Ile	Gln	Arg	Tyr	Phe 200	Ser	Gln	Pro	Thr	Leu 205	Tyr	Ser	Glu		
Pro	Gly 210	Trp	Met	Pro	Gly	Phe 215	Tyr	Arg	Val	Arg	Thr 220	Thr	Val	Asn	Cys		
Glu 225	Ile	Val	Asp	Met	Val 230	Ala	Arg	Ser	Met	Asp 235	Pro	Tyr	Asn	Tyr	Ile 240		
Ala	Thr	Ala	Leu	Gly 245	Asp	Ser	Leu	Glu	Leu 250	Ser	Pro	Phe	Gln	Thr 255	Phe		
Asp	Asn	Thr	Ser 260	Gln	Cys	Thr	Ala	Pro 265	Lys	Arg	Ala	Asp	Met 270	Arg	Val		
Arg	Glu	Val 275	Lys	Asn	Tyr	Lys	Phe 280	Val	Asp	Tyr	Asn	Asn 285	Arg	Gly	Thr		
Ala 290	Pro	Ala	Gly	Gln	Ser	Arg 295	Thr	Phe	Leu	Glu	Thr 300	Pro	Ser	Ala	Thr		
Tyr 305	Ser	Trp	Lys	Thr	Ala 310	Thr	Arg	Gln	Thr	Ala 315	Thr	Cys	Asp	Leu	Val 320		
His	Trp	Lys	Thr	Phe 325	Pro	Arg	Ala	Ile 330	Gln	Thr	Ala	His	Glu	His 335	Ser		
Tyr	His	Phe	Val 340	Ala	Asn	Glu	Val	Thr 345	Ala	Thr	Phe	Asn	Thr 350	Pro	Leu		
Thr	Glu	Val 355	Glu	Asn	Phe	Thr	Ser 360	Thr	Tyr	Ser	Cys	Val 365	Ser	Asp	Gln		
Ile 370	Asn	Lys	Thr	Ile	Ser	Glu 375	Tyr	Ile	Gln	Lys	Leu 380	Asn	Asn	Ser	Tyr		
Val 385	Ala	Ser	Gly	Lys	Thr 390	Gln	Tyr	Phe	Lys	Thr 395	Asp	Gly	Asn	Leu	Tyr 400		
Leu	Ile	Trp	Gln	Pro 405	Leu	Glu	His	Pro	Glu 410	Ile	Glu	Asp	Ile 415	Asp	Glu		
Asp	Ser	Asp	Pro 420	Glu	Pro	Thr	Pro	Ala 425	Pro	Pro	Lys	Ser	Thr 430	Arg	Arg		
Lys	Arg	Glu 435	Ala	Ala	Asp	Asn	Gly 440	Asn	Ser	Thr	Ser	Glu 445	Val	Ser	Lys		
Gly 450	Ser	Glu	Asn	Pro	Leu	Ile 455	Thr	Ala	Gln	Ile	Gln 460	Phe	Ala	Tyr	Asp		
Lys 465	Leu	Thr	Thr	Ser	Val 470	Asn	Asn	Val	Leu	Glu 475	Glu	Leu	Ser	Arg	Ala 480		
Trp	Cys	Arg	Glu	Gln 485	Val	Arg	Asp	Thr	Leu 490	Met	Trp	Tyr	Glu 495	Leu	Ser		
Lys	Val	Asn 500	Pro	Thr	Ser	Val	Met	Ser 505	Ala	Ile	Tyr	Gly	Lys 510	Pro	Val		

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Ala	Ala	Arg	Tyr	Val	Gly	Asp	Ala	Ile	Ser	Val	Thr	Asp	Cys	Ile	Tyr
	515						520					525			
Val	Asp	Gln	Ser	Ser	Val	Asn	Ile	His	Gln	Ser	Leu	Arg	Leu	Gln	His
	530					535					540				
Asp	Lys	Thr	Thr	Cys	Tyr	Ser	Arg	Pro	Arg	Val	Thr	Phe	Lys	Phe	Ile
545					550					555					560
Asn	Ser	Thr	Asp	Pro	Leu	Thr	Gly	Gln	Leu	Gly	Pro	Arg	Lys	Glu	Ile
			565						570					575	
Ile	Leu	Ser	Asn	Thr	Asn	Ile	Glu	Thr	Cys	Lys	Asp	Glu	Ser	Glu	His
			580					585					590		
Tyr	Phe	Ile	Val	Gly	Glu	Tyr	Ile	Tyr	Tyr	Tyr	Lys	Asn	Tyr	Ile	Phe
	595						600					605			
Glu	Glu	Lys	Leu	Asn	Leu	Ser	Ser	Ile	Ala	Thr	Leu	Asp	Thr	Phe	Ile
	610					615					620				
Ala	Leu	Asn	Ile	Ser	Phe	Ile	Glu	Asn	Ile	Asp	Phe	Lys	Thr	Val	Glu
625					630					635					640
Leu	Tyr	Ser	Ser	Thr	Glu	Arg	Lys	Leu	Ala	Ser	Ser	Val	Phe	Asp	Ile
				645					650					655	
Glu	Ser	Met	Phe	Arg	Glu	Tyr	Asn	Tyr	Tyr	Thr	Tyr	Ser	Leu	Ala	Gly
			660					665					670		
Ile	Lys	Lys	Asp	Leu	Asp	Asn	Thr	Ile	Asp	Tyr	Asn	Arg	Asp	Arg	Leu
		675					680					685			
Val	Gln	Asp	Leu	Ser	Asp	Met	Met	Ala	Asp	Leu	Gly	Asp	Ile	Gly	Arg
	690					695					700				
Ser	Val	Val	Asn	Val	Val	Ser	Ser	Val	Val	Thr	Phe	Phe	Ser	Ser	Ile
705				710						715					720
Val	Thr	Gly	Phe	Ile	Lys	Phe	Phe	Thr	Asn	Pro	Leu	Gly	Gly	Ile	Phe
			725						730					735	
Ile	Leu	Leu	Ile	Ile	Gly	Gly	Ile	Ile	Phe	Leu	Val	Val	Val	Leu	Asn
			740					745					750		
Arg	Arg	Asn	Ser	Gln	Phe	His	Asp	Ala	Pro	Ile	Lys	Met	Leu	Tyr	Pro
		755					760					765			
Ser	Val	Glu	Asn	Tyr	Ala	Ala	Arg	Gln	Ala	Pro	Pro	Pro	Tyr	Ser	Ala
	770					775					780				
Ser	Pro	Pro	Ala	Ile	Asp	Lys	Glu	Glu	Ile	Lys	Arg	Ile	Leu	Leu	Gly
785					790					795					800
Met	His	Gln	Val	His	Gln	Glu	Glu	Lys	Glu	Ala	Gln	Lys	Gln	Leu	Thr
				805					810					815	
Asn	Ser	Gly	Pro	Thr	Leu	Trp	Gln	Lys	Ala	Thr	Gly	Phe	Leu	Arg	Asn
			820					825					830		
Arg	Arg	Lys	Gly	Tyr	Ser	Gln	Leu	Pro	Leu	Glu	Asp	Glu	Ser	Thr	Ser
		835					840					845			
Leu															

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 857 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met	Thr	Arg	Arg	Arg	Val	Leu	Ser	Val	Val	Val	Leu	Leu	Ala	Ala	Leu
1					5					10					15

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Ala	Cys	Arg	Leu	Gly	Ala	Gln	Thr	Pro	Glu	Gln	Pro	Ala	Pro	Pro	Ala
			20					25					30		
Thr	Thr	Val	Gln	Pro	Thr	Ala	Thr	Arg	Gln	Gln	Thr	Ser	Phe	Pro	Phe
		35					40					45			
Arg	Val	Cys	Glu	Leu	Ser	Ser	His	Gly	Asp	Leu	Phe	Arg	Phe	Ser	Ser
	50					55					60				
Asp	Ile	Gln	Cys	Pro	Ser	Phe	Gly	Thr	Arg	Glu	Asn	His	Thr	Glu	Gly
65					70					75					80
Leu	Leu	Met	Val	Phe	Lys	Asp	Asn	Ile	Ile	Pro	Tyr	Ser	Phe	Lys	Val
				85					90					95	
Arg	Ser	Tyr	Thr	Lys	Ile	Val	Thr	Asn	Ile	Leu	Ile	Tyr	Asn	Gly	Trp
			100					105					110		
Tyr	Ala	Asp	Ser	Val	Thr	Asn	Arg	His	Glu	Glu	Lys	Phe	Ser	Val	Asp
		115					120					125			
Ser	Tyr	Glu	Thr	Asp	Gln	Met	Asp	Thr	Ile	Tyr	Gln	Cys	Tyr	Asn	Ala
	130					135					140				
Val	Lys	Met	Thr	Lys	Asp	Gly	Leu	Thr	Arg	Val	Tyr	Val	Asp	Arg	Asp
145					150					155					160
Gly	Val	Asn	Ile	Thr	Val	Asn	Leu	Lys	Pro	Thr	Gly	Gly	Leu	Ala	Asn
				165					170					175	
Gly	Val	Arg	Arg	Tyr	Ala	Ser	Gln	Thr	Glu	Leu	Tyr	Asp	Ala	Pro	Gly
			180					185					190		
Trp	Leu	Ile	Trp	Thr	Tyr	Arg	Thr	Arg	Thr	Thr	Val	Asn	Cys	Leu	Ile
		195					200					205			
Thr	Asp	Met	Met	Ala	Lys	Ser	Asn	Ser	Pro	Phe	Asp	Phe	Phe	Val	Thr
	210					215					220				
Thr	Thr	Gly	Gln	Thr	Val	Glu	Met	Ser	Pro	Phe	Tyr	Asp	Gly	Lys	Asn
225					230					235					240
Lys	Glu	Thr	Phe	His	Glu	Arg	Ala	Asp	Ser	Phe	His	Val	Arg	Thr	Asn
				245					250					255	
Tyr	Lys	Ile	Val	Asp	Tyr	Asp	Asn	Arg	Gly	Thr	Asn	Pro	Gln	Gly	Glu
			260					265					270		
Arg	Arg	Ala	Phe	Leu	Asp	Lys	Gly	Thr	Tyr	Thr	Leu	Ser	Trp	Lys	Leu
		275					280					285			
Glu	Asn	Arg	Thr	Ala	Tyr	Cys	Pro	Leu	Gln	His	Trp	Gln	Thr	Phe	Asp
	290					295					300				
Ser	Thr	Ile	Ala	Thr	Glu	Thr	Gly	Lys	Ser	Ile	His	Phe	Val	Thr	Asp
305					310					315					320
Glu	Gly	Thr	Ser	Ser	Phe	Val	Thr	Asn	Thr	Thr	Val	Gly	Ile	Glu	Leu
				325					330					335	
Pro	Asp	Ala	Phe	Lys	Cys	Ile	Glu	Glu	Gln	Val	Asn	Lys	Thr	Met	His
			340					345					350		
Glu	Lys	Tyr	Glu	Ala	Val	Gln	Asp	Arg	Tyr	Thr	Lys	Gly	Gln	Glu	Ala
		355					360					365			
Ile	Thr	Tyr	Phe	Ile	Thr	Ser	Gly	Gly	Leu	Leu	Leu	Ala	Trp	Leu	Pro
	370					375						380			
Leu	Thr	Pro	Arg	Ser	Leu	Ala	Thr	Val	Lys	Asn	Leu	Thr	Glu	Leu	Thr
385					390					395					400
Thr	Pro	Thr	Ser	Ser	Pro	Pro	Ser	Ser	Pro	Ser	Pro	Pro	Ala	Pro	Ser
				405					410					415	
Ala	Ala	Arg	Gly	Ser	Thr	Pro	Ala	Ala	Val	Leu	Arg	Arg	Arg	Arg	Arg
			420					425					430		
Asp	Ala	Gly	Asn	Ala	Thr	Thr	Pro	Val	Pro	Pro	Thr	Ala	Pro	Gly	Lys

435					440					445				
Ser	Leu	Gly	Thr	Leu	Asn	Pro	Ala	Thr	Val	Gln	Ile	Gln	Phe	Ala
450					455					460				
Tyr	Asp	Ser	Leu	Arg	Arg	Gln	Ile	Asn	Arg	Met	Leu	Gly	Asp	Leu
465					470					475				
Arg	Ala	Trp	Cys	Leu	Glu	Gln	Lys	Arg	Gln	Asn	Met	Val	Leu	Arg
485					490					495				
Leu	Thr	Lys	Ile	Asn	Pro	Thr	Thr	Val	Met	Ser	Ser	Ile	Tyr	Gly
500					505					510				
Ala	Val	Ala	Ala	Lys	Arg	Leu	Gly	Asp	Val	Ile	Ser	Val	Ser	Gln
515					520					525				
Val	Pro	Val	Asn	Gln	Ala	Thr	Val	Thr	Leu	Arg	Lys	Ser	Met	Arg
530					535					540				
Pro	Gly	Ser	Glu	Thr	Met	Cys	Tyr	Ser	Arg	Pro	Leu	Val	Ser	Phe
545					550					555				
Phe	Ile	Asn	Asp	Thr	Lys	Thr	Tyr	Glu	Gly	Gln	Leu	Gly	Thr	Asp
565					570					575				
Glu	Ile	Phe	Leu	Thr	Lys	Lys	Met	Thr	Glu	Val	Cys	Gln	Ala	Thr
580					585					590				
Gln	Tyr	Tyr	Phe	Gln	Ser	Gly	Asn	Glu	Ile	His	Val	Tyr	Asn	Asp
595					600					605				
His	His	Phe	Lys	Thr	Ile	Glu	Leu	Asp	Gly	Ile	Ala	Thr	Leu	Gln
610					615					620				
Phe	Ile	Ser	Leu	Asn	Thr	Ser	Leu	Ile	Glu	Asn	Ile	Asp	Phe	Ala
625					630					635				
Leu	Glu	Leu	Tyr	Ser	Arg	Asp	Glu	Gln	Arg	Ala	Ser	Asn	Val	Phe
645					650					655				
Leu	Glu	Gly	Ile	Phe	Arg	Glu	Tyr	Asn	Phe	Gln	Ala	Gln	Asn	Ile
660					665					670				
Gly	Leu	Arg	Lys	Asp	Leu	Asp	Asn	Ala	Val	Ser	Asn	Gly	Arg	Asn
675					680					685				
Phe	Val	Asp	Gly	Leu	Gly	Glu	Leu	Met	Asp	Ser	Leu	Gly	Ser	Val
690					695					700				
Gln	Ser	Ile	Thr	Asn	Leu	Val	Ser	Thr	Val	Gly	Gly	Leu	Phe	Ser
705					710					715				
Leu	Val	Ser	Gly	Phe	Ile	Ser	Phe	Phe	Lys	Asn	Pro	Phe	Gly	Gly
725					730					735				
Leu	Ile	Leu	Val	Leu	Val	Ala	Gly	Val	Val	Ile	Leu	Val	Ile	Ser
740					745					750				
Thr	Arg	Arg	Thr	Arg	Gln	Met	Ser	Gln	Gln	Pro	Val	Gln	Met	Leu
755					760					765				
Pro	Gly	Ile	Asp	Glu	Leu	Ala	Gln	Gln	His	Ala	Ser	Gly	Glu	Gly
770					775					780				
Gly	Ile	Asn	Pro	Ile	Ser	Lys	Thr	Glu	Leu	Gln	Ala	Ile	Met	Leu
785					790					795				
Leu	His	Glu	Gln	Asn	Gln	Glu	Gln	Lys	Arg	Ala	Ala	Gln	Arg	Ala
805					810					815				
Gly	Pro	Ser	Val	Ala	Ser	Arg	Ala	Leu	Gln	Ala	Ala	Arg	Asp	Arg
820					825					830				
Pro	Gly	Leu	Arg	Arg	Arg	Arg	Tyr	His	Asp	Pro	Glu	Thr	Ala	Ala
835					840					845				
Leu	Leu	Gly	Glu	Ala	Glu	Thr	Glu	Phe						
850					855									

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(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 907 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

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Met Glu Ser Arg Ile Trp Cys Leu Val Val Cys Val Asn Leu Cys Ile
 1           5           10          15

Val Cys Leu Gly Ala Ala Val Ser Ser Ser Thr Arg Gly Thr Ser
          20          25          30

Ala Thr His Ser His His Ser Ser His Thr Thr Ser Ala Ala His Ser
          35          40          45

Arg Ser Gly Ser Val Ser Gln Arg Val Thr Ser Ser Gln Thr Val Ser
          50          55          60

His Gly Val Asn Glu Thr Ile Tyr Asn Thr Thr Leu Lys Tyr Gly Asp
65          70          75          80

Val Val Gly Val Asn Thr Thr Lys Tyr Pro Tyr Arg Val Cys Ser Met
          85          90          95

Ala Gln Gly Thr Asp Leu Ile Arg Phe Glu Arg Asn Ile Val Cys Thr
          100         105         110

Ser Met Lys Pro Ile Asn Glu Asp Leu Asp Glu Gly Ile Met Val Val
          115         120         125

Tyr Lys Arg Asn Ile Val Ala His Thr Phe Lys Val Arg Val Tyr Gln
          130         135         140

Lys Val Leu Thr Phe Arg Arg Ser Tyr Ala Tyr Ile His Thr Thr Tyr
          145         150         155         160

Leu Leu Gly Ser Asn Thr Glu Tyr Val Ala Pro Pro Met Trp Glu Ile
          165         170         175

His His Ile Asn Ser His Ser Gln Cys Tyr Ser Ser Tyr Ser Arg Val
          180         185         190

Ile Ala Gly Thr Val Phe Val Ala Tyr His Arg Asp Ser Tyr Glu Asn
          195         200         205

Lys Thr Met Gln Leu Met Pro Asp Asp Tyr Ser Asn Thr His Ser Thr
          210         215         220

Arg Tyr Val Thr Val Lys Asp Gln Trp His Ser Arg Gly Ser Thr Trp
          225         230         235         240

Leu Tyr Arg Glu Thr Cys Asn Leu Asn Cys Met Val Thr Ile Thr Thr
          245         250         255

Ala Arg Ser Lys Tyr Pro Tyr His Phe Phe Ala Thr Ser Thr Gly Asp
          260         265         270

Val Val Asp Ile Ser Pro Phe Tyr Asn Gly Thr Asn Arg Asn Ala Ser
          275         280         285

Tyr Phe Gly Glu Asn Ala Asp Lys Phe Phe Ile Phe Pro Asn Tyr Thr
          290         295         300

Ile Val Ser Asp Phe Gly Arg Pro Asn Ser Ala Leu Glu Thr His Arg
          305         310         315         320

Leu Val Ala Phe Leu Glu Arg Ala Asp Ser Val Ile Ser Trp Asp Ile
          325         330         335

Gln Asp Glu Lys Asn Val Thr Cys Gln Leu Thr Phe Trp Glu Ala Ser
          340         345         350

Glu Arg Thr Ile Arg Ser Glu Ala Glu Asp Ser Tyr His Phe Ser Ser

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355						360						365					
Ala	Lys	Met	Thr	Ala	Thr	Phe	Leu	Ser	Lys	Lys	Gln	Glu	Val	Asn	Met		
	370					375					380						
Ser	Asp	Ser	Ala	Leu	Asp	Cys	Val	Arg	Asp	Glu	Ala	Ile	Asn	Lys	Leu		
385					390					395					400		
Gln	Gln	Ile	Phe	Asn	Thr	Ser	Tyr	Asn	Gln	Thr	Tyr	Glu	Lys	Tyr	Gly		
				405					410					415			
Asn	Val	Ser	Val	Phe	Glu	Thr	Thr	Gly	Gly	Leu	Val	Val	Phe	Trp	Gln		
			420					425					430				
Gly	Ile	Lys	Gln	Lys	Ser	Leu	Val	Glu	Leu	Glu	Arg	Leu	Ala	Asn	Arg		
	435						440					445					
Ser	Ser	Leu	Asn	Leu	Thr	His	Asn	Arg	Thr	Lys	Arg	Ser	Thr	Asp	Gly		
450						455					460						
Asn	Asn	Ala	Thr	His	Leu	Ser	Asn	Met	Glu	Ser	Val	His	Asn	Leu	Val		
465					470					475					480		
Tyr	Ala	Gln	Leu	Gln	Phe	Thr	Tyr	Asp	Thr	Leu	Arg	Gly	Tyr	Ile	Asn		
				485					490					495			
Arg	Ala	Leu	Ala	Gln	Ile	Ala	Glu	Ala	Trp	Cys	Val	Asp	Gln	Arg	Arg		
			500					505					510				
Thr	Leu	Glu	Val	Phe	Lys	Glu	Leu	Ser	Lys	Ile	Asn	Pro	Ser	Ala	Ile		
	515						520					525					
Leu	Ser	Ala	Ile	Tyr	Asn	Lys	Pro	Ile	Ala	Ala	Arg	Phe	Met	Gly	Asp		
530					535						540						
Val	Leu	Gly	Leu	Ala	Ser	Cys	Val	Thr	Ile	Asn	Gln	Thr	Ser	Val	Lys		
545					550					555					560		
Val	Leu	Arg	Asp	Met	Asn	Val	Lys	Glu	Ser	Pro	Gly	Arg	Cys	Tyr	Ser		
				565					570					575			
Arg	Pro	Val	Val	Ile	Phe	Asn	Phe	Ala	Asn	Ser	Ser	Tyr	Val	Gln	Tyr		
			580					585					590				
Gly	Gln	Leu	Gly	Glu	Asp	Asn	Glu	Ile	Leu	Leu	Gly	Asn	His	Arg	Thr		
	595						600					605					
Glu	Glu	Cys	Gln	Leu	Pro	Ser	Leu	Lys	Ile	Phe	Ile	Ala	Gly	Asn	Ser		
610						615					620						
Ala	Tyr	Glu	Tyr	Val	Asp	Tyr	Leu	Phe	Lys	Arg	Met	Ile	Asp	Leu	Ser		
625					630					635					640		
Ser	Ile	Ser	Thr	Val	Asp	Ser	Met	Ile	Ala	Leu	Asp	Ile	Asp	Pro	Leu		
				645					650					655			
Glu	Asn	Thr	Asp	Phe	Arg	Val	Leu	Glu	Leu	Tyr	Ser	Gln	Lys	Glu	Leu		
			660					665					670				
Arg	Ser	Ser	Asn	Val	Phe	Asp	Leu	Glu	Glu	Ile	Met	Arg	Glu	Phe	Asn		
	675						680					685					
Ser	Tyr	Lys	Gln	Arg	Val	Lys	Tyr	Val	Glu	Asp	Lys	Val	Val	Asp	Pro		
690						695					700						
Leu	Pro	Pro	Tyr	Leu	Lys	Gly	Leu	Asp	Asp	Leu	Met	Ser	Gly	Leu	Gly		
705					710					715					720		
Ala	Ala	Gly	Lys	Ala	Val	Gly	Val	Ala	Ile	Gly	Ala	Val	Gly	Gly	Ala		
				725					730					735			
Val	Ala	Ser	Val	Val	Glu	Gly	Val	Ala	Thr	Phe	Leu	Lys	Asn	Pro	Phe		
			740					745					750				
Gly	Ala	Phe	Thr	Ile	Ile	Leu	Val	Ala	Ile	Ala	Val	Val	Ile	Ile	Ile		
	755						760					765					
Tyr	Leu	Ile	Tyr	Thr	Arg	Gln	Arg	Arg	Leu	Cys	Met	Gln	Pro	Leu	Gln		
770						775					780						

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Asn	Leu	Phe	Pro	Tyr	Leu	Val	Ser	Ala	Asp	Gly	Thr	Thr	Val	Thr	Ser
785					790					795					800
Gly	Asn	Thr	Lys	Asp	Thr	Ser	Leu	Gln	Ala	Pro	Pro	Ser	Tyr	Glu	Glu
			805						810					815	
Ser	Val	Tyr	Asn	Ser	Gly	Arg	Lys	Gly	Pro	Gly	Pro	Pro	Ser	Ser	Asp
			820					825					830		
Ala	Ser	Thr	Ala	Ala	Pro	Pro	Tyr	Thr	Asn	Glu	Gln	Ala	Tyr	Gln	Met
		835					840					845			
Leu	Leu	Ala	Leu	Val	Arg	Leu	Asp	Ala	Glu	Gln	Arg	Ala	Gln	Gln	Asn
	850					855					860				
Gly	Thr	Asp	Ser	Leu	Asp	Gly	Gln	Thr	Gly	Thr	Gln	Asp	Lys	Gly	Gln
865				870					875						880
Lys	Pro	Asn	Leu	Leu	Asp	Arg	Leu	Arg	His	Arg	Lys	Asn	Gly	Tyr	Arg
			885					890						895	
His	Leu	Lys	Asp	Ser	Asp	Glu	Glu	Glu	Asn	Val					
			900					905							

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 830 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met	Ser	Lys	Met	Val	Val	Leu	Phe	Leu	Ala	Val	Phe	Leu	Met	Asn	Ser
1			5						10				15		
Val	Leu	Met	Ile	Tyr	Cys	Asp	Pro	Asp	His	Tyr	Ile	Arg	Ala	Gly	Tyr
		20					25					30			
Asn	His	Lys	Tyr	Pro	Phe	Arg	Ile	Cys	Ser	Ile	Ala	Lys	Gly	Thr	Asp
		35				40						45			
Leu	Met	Arg	Phe	Asp	Arg	Asp	Ile	Ser	Cys	Ser	Pro	Tyr	Lys	Ser	Asn
	50				55						60				
Ala	Lys	Met	Ser	Glu	Gly	Phe	Phe	Ile	Ile	Tyr	Lys	Thr	Asn	Ile	Glu
65				70					75						80
Thr	Tyr	Thr	Phe	Pro	Val	Arg	Thr	Tyr	Lys	Lys	Glu	Leu	Thr	Phe	Gln
			85					90					95		
Ser	Ser	Tyr	Arg	Asp	Val	Gly	Val	Val	Tyr	Phe	Leu	Asp	Arg	Thr	Val
		100					105						110		
Met	Gly	Leu	Ala	Met	Pro	Val	Tyr	Glu	Ala	Asn	Leu	Val	Asn	Ser	His
	115					120						125			
Ala	Gln	Cys	Tyr	Ser	Ala	Val	Ala	Met	Lys	Arg	Pro	Asp	Gly	Thr	Val
	130				135						140				
Phe	Ser	Ala	Phe	His	Glu	Asp	Asn	Asn	Lys	Asn	Asn	Thr	Leu	Asn	Leu
145				150					155						160
Phe	Pro	Leu	Asn	Phe	Lys	Ser	Ile	Thr	Asn	Lys	Arg	Phe	Ile	Thr	Thr
			165					170					175		
Lys	Glu	Pro	Tyr	Phe	Ala	Arg	Gly	Pro	Leu	Trp	Leu	Tyr	Ser	Thr	Ser
		180					185					190			
Thr	Ser	Leu	Asn	Cys	Ile	Val	Thr	Glu	Ala	Thr	Ala	Lys	Ala	Lys	Tyr
	195					200					205				
Pro	Phe	Ser	Tyr	Phe	Ala	Leu	Thr	Thr	Gly	Glu	Ile	Val	Glu	Gly	Ser
	210					215					220				
Pro	Phe	Phe	Asn	Gly	Ser	Asn	Gly	Lys	His	Phe	Ala	Glu	Pro	Leu	Glu

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225	230	235	240
Lys Leu Thr Ile Leu Glu Asn Tyr Thr Met Ile Glu Asp Leu Met Asn	245	250	255
Gly Met Asn Gly Ala Thr Thr Leu Val Arg Lys Ile Ala Phe Leu Glu	260	265	270
Lys Ala Asp Thr Leu Phe Ser Trp Glu Ile Lys Glu Glu Asn Glu Ser	275	280	285
Val Cys Met Leu Lys His Trp Thr Thr Val Thr His Gly Leu Arg Ala	290	295	300
Glu Thr Asp Glu Thr Tyr His Phe Ile Ser Lys Glu Leu Thr Ala Ala	305	310	315
Phe Val Ala Pro Lys Glu Ser Leu Asn Leu Thr Asp Pro Lys Gln Thr	325	330	335
Cys Ile Lys Asp Glu Phe Glu Lys Ile Ile Asn Glu Val Tyr Met Ser	340	345	350
Asp Tyr Asn Asp Thr Tyr Ser Met Asn Gly Ser Tyr Gln Ile Phe Lys	355	360	365
Thr Thr Gly Asp Leu Ile Leu Ile Trp Gln Pro Leu Val Gln Lys Ser	370	375	380
Leu Met Phe Leu Glu Gln Gly Ser Glu Lys Ile Arg Arg Arg Arg Asp	385	390	395
Val Val Asp Val Lys Ser Arg His Asp Ile Leu Tyr Val Gln Leu Gln	405	410	415
Tyr Leu Tyr Asp Thr Leu Lys Asp Tyr Ile Asn Asp Ala Leu Gly Asn	420	425	430
Leu Ala Glu Ser Trp Cys Leu Asp Gln Lys Arg Thr Ile Thr Met Leu	435	440	445
His Glu Leu Ser Lys Ile Ser Pro Ser Ser Ile Val Ser Glu Val Tyr	450	455	460
Gly Arg Pro Ile Ser Ala Gln Leu His Gly Asp Val Leu Ala Ile Ser	465	470	475
Lys Cys Ile Glu Val Asn Gln Ser Ser Val Gln Leu His Lys Ser Met	485	490	495
Arg Val Val Asp Ala Lys Gly Val Arg Ser Glu Thr Met Cys Tyr Asn	500	505	510
Arg Pro Leu Val Thr Phe Ser Phe Val Asn Ser Thr Pro Glu Val Val	515	520	525
Pro Gly Gln Leu Gly Leu Asp Asn Glu Ile Leu Leu Gly Asp His Arg	530	535	540
Thr Glu Glu Cys Glu Ile Pro Ser Thr Lys Ile Phe Leu Ser Gly Asn	545	550	555
His Ala His Val Tyr Thr Asp Tyr Thr His Thr Asn Ser Thr Pro Ile	565	570	575
Glu Asp Ile Glu Val Leu Asp Ala Phe Ile Arg Leu Lys Ile Asp Pro	580	585	590
Leu Glu Asn Ala Asp Phe Lys Val Leu Asp Leu Tyr Ser Pro Asp Glu	595	600	605
Leu Ser Arg Ala Asn Val Phe Asp Leu Glu Asn Ile Leu Arg Glu Tyr	610	615	620
Asn Ser Tyr Lys Ser Ala Leu Tyr Thr Ile Glu Ala Lys Ile Ala Thr	625	630	635
Asn Thr Pro Ser Tyr Val Asn Gly Ile Asn Ser Phe Leu Gln Gly Leu	645	650	655

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Gly Ala Ile Gly Thr Gly Leu Gly Ser Val Ile Ser Val Thr Ala Gly
660 665 670

Ala Leu Gly Asp Ile Val Gly Gly Val Val Ser Phe Leu Lys Asn Pro
675 680 685

Phe Gly Gly Gly Leu Met Leu Ile Leu Ala Ile Val Val Val Val Ile
690 695 700

Ile Ile Val Val Phe Val Arg Gln Arg His Val Leu Ser Lys Pro Ile
705 710 715 720

Asp Met Met Phe Pro Tyr Ala Thr Asn Pro Val Thr Thr Val Ser Ser
725 730 735

Val Thr Gly Thr Thr Val Val Lys Thr Pro Ser Val Lys Asp Val Asp
740 745 750

Gly Gly Thr Ser Val Ala Val Ser Glu Lys Glu Glu Gly Met Ala Asp
755 760 765

Val Ser Gly Gln Val Ser Asp Asp Glu Tyr Ser Gln Glu Ala Ala Leu
770 775 780

Lys Met Leu Lys Ala Ile Lys Ser Leu Asp Glu Ser Tyr Arg Arg Lys
785 790 795 800

Pro Ser Ser Ser Glu Ser His Ala Ser Lys Pro Ser Leu Ile Asp Arg
805 810 815

Ile Arg Tyr Arg Gly Tyr Lys Ser Val Asn Val Glu Glu Ala
820 825 830

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 868 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Met Phe Val Thr Ala Val Val Ser Val Ser Pro Ser Ser Phe Tyr Glu
1 5 10 15

Ser Leu Gln Val Glu Pro Thr Gln Ser Glu Asp Ile Thr Arg Ser Ala
20 25 30

His Leu Gly Asp Gly Asp Glu Ile Arg Glu Ala Ile His Lys Ser Gln
35 40 45

Asp Ala Glu Thr Lys Pro Thr Phe Tyr Val Cys Pro Pro Pro Thr Gly
50 55 60

Ser Thr Ile Val Arg Leu Glu Pro Thr Arg Thr Cys Pro Asp Tyr His
65 70 75 80

Leu Gly Lys Asn Phe Thr Glu Gly Ile Ala Val Val Tyr Lys Glu Asn
85 90 95

Ile Ala Ala Tyr Lys Phe Lys Ala Thr Val Tyr Tyr Lys Asp Val Ile
100 105 110

Val Ser Thr Ala Trp Ala Gly Ser Ser Tyr Thr Gln Ile Thr Asn Arg
115 120 125

Tyr Ala Asp Arg Val Pro Ile Pro Val Ser Glu Ile Thr Asp Thr Ile
130 135 140

Asp Lys Phe Gly Lys Cys Ser Ser Lys Ala Thr Tyr Val Arg Asn Asn
145 150 155 160

His Lys Val Glu Ala Phe Asn Glu Asp Lys Asn Pro Gln Asp Met Pro
165 170 175

Leu Ile Ala Ser Lys Tyr Asn Ser Val Gly Ser Lys Ala Trp His Thr

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180							185					190				
Thr	Asn	Asp	Thr	Tyr	Met	Val	Ala	Gly	Thr	Pro	Gly	Thr	Tyr	Arg	Thr	
		195					200					205				
Gly	Thr	Ser	Val	Asn	Cys	Ile	Ile	Glu	Glu	Val	Glu	Ala	Arg	Ser	Ile	
	210					215					220					
Phe	Pro	Tyr	Asp	Ser	Phe	Gly	Leu	Ser	Thr	Gly	Asp	Ile	Ile	Tyr	Met	
225					230					235					240	
Ser	Pro	Phe	Phe	Gly	Leu	Arg	Asp	Gly	Ala	Tyr	Arg	Glu	His	Ser	Asn	
				245					250					255		
Tyr	Ala	Met	Asp	Arg	Phe	His	Gln	Phe	Glu	Gly	Tyr	Arg	Gln	Arg	Asp	
			260					265					270			
Leu	Asp	Thr	Arg	Ala	Leu	Leu	Glu	Pro	Ala	Ala	Arg	Asn	Phe	Leu	Val	
	275						280					285				
Thr	Pro	His	Leu	Thr	Val	Gly	Trp	Asn	Trp	Lys	Pro	Lys	Arg	Thr	Glu	
	290					295					300					
Val	Cys	Ser	Leu	Val	Lys	Trp	Arg	Glu	Val	Glu	Asp	Val	Val	Arg	Asp	
305					310					315					320	
Glu	Tyr	Ala	His	Asn	Phe	Arg	Phe	Thr	Met	Lys	Thr	Leu	Ser	Thr	Thr	
				325					330					335		
Phe	Ile	Ser	Glu	Thr	Asn	Glu	Phe	Asn	Leu	Asn	Gln	Ile	His	Leu	Ser	
		340						345					350			
Gln	Cys	Val	Lys	Glu	Glu	Ala	Arg	Ala	Ile	Ile	Asn	Arg	Ile	Tyr	Thr	
		355					360					365				
Thr	Arg	Tyr	Asn	Ser	Ser	His	Val	Arg	Thr	Gly	Asp	Ile	Gln	Thr	Tyr	
	370					375					380					
Leu	Ala	Arg	Gly	Gly	Phe	Val	Val	Val	Phe	Gln	Pro	Leu	Leu	Ser	Asn	
385					390					395					400	
Ser	Leu	Ala	Arg	Leu	Tyr	Leu	Gln	Glu	Leu	Val	Arg	Glu	Asn	Thr	Asn	
				405					410					415		
His	Ser	Pro	Gln	Lys	His	Pro	Thr	Arg	Asn	Thr	Arg	Ser	Arg	Arg	Ser	
			420					425					430			
Val	Pro	Val	Glu	Leu	Arg	Ala	Asn	Arg	Thr	Ile	Thr	Thr	Thr	Ser	Ser	
		435					440					445				
Val	Glu	Phe	Ala	Met	Leu	Gln	Phe	Thr	Tyr	Asp	His	Ile	Gln	Glu	His	
	450					455					460					
Val	Asn	Glu	Met	Leu	Ala	Arg	Ile	Ser	Ser	Ser	Trp	Cys	Gln	Leu	Gln	
465					470					475					480	
Asn	Arg	Glu	Arg	Ala	Leu	Trp	Ser	Gly	Leu	Phe	Pro	Ile	Asn	Pro	Ser	
				485					490					495		
Ala	Leu	Ala	Ser	Thr	Ile	Leu	Asp	Gln	Arg	Val	Lys	Ala	Arg	Ile	Leu	
			500					505					510			
Gly	Asp	Val	Ile	Ser	Val	Ser	Asn	Cys	Pro	Glu	Leu	Gly	Ser	Asp	Thr	
		515					520					525				
Arg	Ile	Ile	Leu	Gln	Asn	Ser	Met	Arg	Val	Ser	Gly	Ser	Thr	Thr	Arg	
	530					535					540					
Cys	Tyr	Ser	Arg	Pro	Leu	Ile	Ser	Ile	Val	Ser	Leu	Asn	Gly	Ser	Gly	
545					550					555					560	
Thr	Val	Glu	Gly	Gln	Leu	Gly	Thr	Asp	Asn	Glu	Leu	Ile	Met	Ser	Arg	
				565					570					575		
Asp	Leu	Leu	Glu	Pro	Cys	Val	Ala	Asn	His	Lys	Arg	Tyr	Phe	Leu	Phe	
			580					585					590			
Gly	His	His	Tyr	Val	Tyr	Tyr	Glu	Asp	Tyr	Arg	Tyr	Val	Arg	Glu	Ile	
			595				600					605				

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Ala Val His Asp Val Gly Met Ile Ser Thr Tyr Val Asp Leu Asn Leu
610 615 620

Thr Leu Leu Lys Asp Arg Glu Phe Met Pro Leu Gln Val Tyr Thr Arg
625 630 635 640

Asp Glu Leu Arg Asp Thr Gly Leu Leu Asp Tyr Ser Glu Ile Gln Arg
645 650 655

Arg Asn Gln Met His Ser Leu Arg Phe Tyr Asp Ile Asp Lys Val Val
660 665 670

Gln Tyr Asp Ser Gly Thr Ala Ile Met Gln Gly Met Ala Gln Phe Phe
675 680 685

Gln Gly Leu Gly Thr Ala Gly Gln Ala Val Gly His Val Val Leu Gly
690 695 700

Ala Thr Gly Ala Leu Leu Ser Thr Val His Gly Phe Thr Thr Phe Leu
705 710 715 720

Ser Asn Pro Phe Gly Ala Leu Ala Val Gly Leu Leu Val Leu Ala Gly
725 730 735

Leu Val Ala Ala Phe Phe Ala Tyr Arg Tyr Val Leu Lys Leu Lys Thr
740 745 750

Ser Pro Met Lys Ala Leu Tyr Pro Leu Thr Thr Lys Gly Leu Lys Gln
755 760 765

Leu Pro Glu Gly Met Asp Pro Phe Ala Glu Lys Pro Asn Ala Thr Asp
770 775 780

Thr Pro Ile Glu Glu Ile Gly Asp Ser Gln Asn Thr Glu Pro Ser Val
785 790 795 800

Asn Ser Gly Phe Asp Pro Asp Lys Phe Arg Glu Ala Gln Glu Met Ile
805 810 815

Lys Tyr Met Thr Leu Val Ser Ala Ala Glu Arg Gln Glu Ser Lys Ala
820 825 830

Arg Lys Lys Asn Lys Thr Ser Ala Leu Leu Thr Ser Arg Leu Thr Gly
835 840 845

Leu Ala Leu Arg Asn Arg Arg Gly Tyr Ser Arg Val Arg Thr Glu Asn
850 855 860

Val Thr Gly Val
865

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 903 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Met Arg Gln Gly Ala Ala Arg Gly Cys Arg Trp Phe Val Val Trp Ala
1 5 10 15

Leu Leu Gly Leu Thr Leu Gly Val Leu Val Ala Ser Ala Ala Pro Ser
20 25 30

Ser Pro Gly Thr Pro Gly Val Ala Ala Ala Thr Gln Ala Ala Asn Gly
35 40 45

Gly Pro Ala Thr Pro Ala Pro Pro Ala Pro Gly Pro Ala Pro Thr Gly
50 55 60

Asp Thr Lys Pro Lys Lys Asn Lys Lys Pro Lys Asn Pro Pro Pro Pro
65 70 75 80

Arg Pro Ala Gly Asp Asn Ala Thr Val Ala Ala Gly His Ala Thr Leu

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85								90				95			
Arg	Glu	His	Leu 100	Arg	Asp	Ile	Lys	Ala 105	Glu	Asn	Thr	Asp	Ala 110	Asn	Phe
Tyr	Val	Cys 115	Pro	Pro	Pro	Thr	Gly 120	Ala	Thr	Val	Val	Gln 125	Phe	Glu	Gln
Pro	Arg 130	Arg	Cys	Pro	Thr	Arg 135	Pro	Glu	Gly	Gln	Asn 140	Tyr	Thr	Glu	Gly
Ile 145	Ala	Val	Val	Phe	Lys 150	Glu	Asn	Ile	Ala	Pro 155	Tyr	Lys	Phe	Lys	Ala 160
Thr	Met	Tyr	Tyr	Lys 165	Asp	Val	Thr	Val	Ser 170	Gln	Val	Trp	Phe	Gly 175	His
Arg	Tyr	Ser	Gln 180	Phe	Met	Gly	Ile	Phe 185	Glu	Asp	Arg	Ala	Pro 190	Val	Pro
Phe	Glu	Glu 195	Val	Ile	Asp	Lys	Ile 200	Asn	Ala	Lys	Gly	Val 205	Cys	Arg	Ser
Thr	Ala 210	Lys	Tyr	Val	Arg	Asn 215	Asn	Leu	Glu	Thr	Thr 220	Ala	Phe	His	Arg
Asp 225	Asp	His	Glu	Thr	Asp 230	Met	Glu	Leu	Lys	Pro 235	Ala	Asn	Ala	Ala	Thr 240
Arg	Thr	Ser	Arg	Gly 245	Trp	His	Thr	Thr	Asp 250	Leu	Lys	Tyr	Asn 255	Pro	Ser
Arg	Val	Glu	Ala 260	Phe	His	Arg	Tyr	Gly 265	Thr	Thr	Val	Asn 270	Cys	Ile	Val
Glu	Glu	Val 275	Asp	Ala	Arg	Ser	Val 280	Tyr	Pro	Tyr	Asp	Glu 285	Phe	Val	Leu
Ala	Thr 290	Gly	Asp	Phe	Val	Tyr 295	Met	Ser	Pro	Phe	Tyr 300	Gly	Tyr	Arg	Glu
Gly 305	Ser	His	Thr	Glu	His 310	Thr	Ser	Tyr	Ala	Ala 315	Asp	Arg	Phe	Lys	Gln 320
Val	Asp	Gly	Phe	Tyr 325	Ala	Arg	Asp	Leu	Thr 330	Thr	Lys	Ala	Arg	Ala 335	Thr
Ala	Pro	Thr	Thr 340	Arg	Asn	Leu	Leu	Thr 345	Thr	Pro	Lys	Phe	Thr 350	Val	Ala
Trp	Asp	Trp 355	Val	Pro	Lys	Arg	Pro 360	Ser	Val	Cys	Thr	Met 365	Thr	Lys	Trp
Gln	Glu 370	Val	Asp	Glu	Met	Leu 375	Arg	Ser	Glu	Tyr	Gly 380	Gly	Ser	Phe	Arg
Phe 385	Ser	Ser	Asp	Ala	Ile 390	Ser	Thr	Thr	Phe	Thr 395	Thr	Asn	Leu	Thr	Glu 400
Tyr	Pro	Leu	Ser	Arg 405	Val	Asp	Leu	Gly	Asp 410	Cys	Ile	Gly	Lys	Asp 415	Ala
Arg	Asp	Ala	Met 420	Asp	Arg	Ile	Phe	Ala 425	Arg	Arg	Tyr	Asn 430	Ala	Thr	His
Ile	Lys 435	Val	Gly	Gln	Pro	Gln	Tyr 440	Tyr	Leu	Ala	Asn 445	Gly	Gly	Phe	Leu
Ile 450	Ala	Tyr	Gln	Pro	Leu	Leu 455	Ser	Asn	Thr	Leu	Ala 460	Glu	Leu	Tyr	Val
Arg 465	Glu	His	Leu	Arg	Glu 470	Gln	Ser	Arg	Lys	Pro 475	Pro	Asn	Pro	Thr	Pro 480
Pro	Pro	Pro	Gly 485	Ala	Ser	Ala	Asn	Ala	Ser 490	Val	Glu	Arg	Ile	Lys 495	Thr
Thr	Ser	Ser	Ile 500	Glu	Phe	Ala	Arg	Leu 505	Gln	Phe	Thr	Tyr	Asn 510	His	Ile

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Gln	Arg	His	Val	Asn	Asp	Met	Leu	Gly	Arg	Val	Ala	Ile	Ala	Trp	Cys
	515						520					525			
Glu	Leu	Gln	Asn	His	Glu	Leu	Thr	Leu	Trp	Asn	Glu	Ala	Arg	Lys	Leu
	530					535					540				
Asn	Pro	Asn	Ala	Ile	Ala	Ser	Ala	Thr	Val	Gly	Arg	Arg	Val	Ser	Ala
545					550					555					560
Arg	Met	Leu	Gly	Asp	Val	Met	Ala	Val	Ser	Thr	Cys	Val	Pro	Val	Ala
				565					570						575
Ala	Asp	Asn	Val	Ile	Val	Gln	Asn	Ser	Met	Arg	Ile	Ser	Ser	Arg	Pro
			580					585							590
Gly	Ala	Cys	Tyr	Ser	Arg	Pro	Leu	Val	Ser	Phe	Arg	Tyr	Glu	Asp	Gln
		595					600					605			
Gly	Pro	Leu	Val	Glu	Gly	Gln	Val	Gly	Glu	Asn	Asn	Glu	Leu	Arg	Leu
	610					615						620			
Thr	Arg	Asp	Ala	Ile	Glu	Pro	Cys	Thr	Val	Gly	His	Arg	Arg	Tyr	Phe
625					630					635					640
Thr	Phe	Gly	Gly	Gly	Tyr	Val	Tyr	Phe	Glu	Glu	Tyr	Ala	Tyr	Ser	His
				645					650					655	
Gln	Leu	Ser	Arg	Ala	Asp	Ile	Thr	Thr	Val	Ser	Thr	Phe	Ile	Asp	Leu
			660					665					670		
Asn	Ile	Thr	Met	Leu	Glu	Asp	His	Glu	Phe	Val	Pro	Leu	Glu	Val	Tyr
		675					680					685			
Thr	Arg	His	Glu	Ile	Lys	Asp	Ser	Gly	Leu	Leu	Asp	Tyr	Thr	Glu	Val
	690					695					700				
Gln	Arg	Arg	Asn	Gln	Leu	His	Asp	Leu	Arg	Phe	Ala	Asp	Ile	Asp	Thr
705				710						715					720
Val	Ile	His	Ala	Asp	Ala	Asn	Ala	Ala	Met	Phe	Ala	Gly	Leu	Gly	Ala
				725					730					735	
Phe	Phe	Glu	Gly	Met	Gly	Asp	Leu	Gly	Arg	Ala	Val	Gly	Lys	Val	Val
		740						745					750		
Met	Gly	Ile	Val	Gly	Gly	Val	Val	Ser	Ala	Val	Ser	Gly	Val	Ser	Ser
	755					760						765			
Phe	Met	Ser	Asn	Pro	Phe	Gly	Ala	Leu	Ala	Val	Gly	Leu	Leu	Val	Leu
	770					775					780				
Ala	Gly	Leu	Ala	Ala	Ala	Phe	Phe	Ala	Phe	Arg	Tyr	Val	Met	Arg	Leu
785				790					795						800
Gln	Ser	Asn	Pro	Met	Lys	Ala	Leu	Tyr	Pro	Leu	Thr	Thr	Lys	Glu	Leu
			805						810					815	
Lys	Asn	Pro	Thr	Asn	Pro	Asp	Ala	Ser	Gly	Glu	Gly	Glu	Glu	Gly	Gly
		820						825					830		
Asp	Phe	Asp	Glu	Ala	Lys	Leu	Ala	Glu	Ala	Arg	Glu	Met	Ile	Arg	Tyr
	835						840					845			
Met	Ala	Leu	Val	Ser	Ala	Met	Glu	Arg	Thr	Glu	His	Lys	Ala	Lys	Lys
	850					855					860				
Lys	Gly	Thr	Ser	Ala	Leu	Leu	Ser	Ala	Lys	Val	Thr	Asp	Met	Val	Met
865				870						875					880
Arg	Lys	Arg	Arg	Asn	Thr	Asn	Tyr	Thr	Gln	Val	Pro	Asn	Lys	Asp	Gly
				885					890					895	
Asp	Ala	Asp	Glu	Asp	Asp	Leu									
			900												

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 885 amino acids

-continued

(B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

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Met Arg Pro Arg Gly Thr Pro Pro Ser Phe Leu Pro Leu Pro Val Leu
 1           5           10           15
Leu Ala Leu Ala Val Ile Ala Ala Ala Gly Arg Ala Ala Pro Ala Ala
      20           25           30
Ala Ala Ala Pro Thr Ala Asp Pro Ala Ala Thr Pro Ala Leu Pro Glu
      35           40           45
Asp Glu Glu Val Pro Asp Glu Asp Gly Glu Gly Val Ala Thr Pro Ala
 50           55           60
Pro Ala Ala Asn Ala Ser Val Glu Ala Gly Arg Ala Thr Leu Arg Glu
 65           70           75           80
Asp Leu Arg Glu Ile Lys Ala Arg Asp Gly Asp Ala Thr Phe Tyr Val
      85           90           95
Cys Pro Pro Pro Thr Gly Ala Thr Val Val Gln Phe Glu Gln Pro Arg
      100          105          110
Pro Cys Pro Arg Ala Pro Asp Gly Gln Asn Tyr Thr Glu Gly Ile Ala
      115          120          125
Val Val Phe Lys Glu Asn Ile Ala Pro Tyr Lys Phe Lys Ala Thr Met
      130          135          140
Tyr Tyr Lys Asp Val Thr Val Ser Gln Val Trp Phe Gly His Arg Tyr
      145          150          155          160
Ser Gln Phe Met Gly Ile Phe Glu Asp Arg Ala Pro Val Pro Phe Glu
      165          170          175
Glu Val Met Asp Lys Ile Asn Ala Lys Gly Val Cys Arg Ser Thr Ala
      180          185          190
Lys Tyr Val Arg Asn Asn Met Glu Ser Thr Ala Phe His Arg Asp Asp
      195          200          205
His Glu Ser Asp Met Ala Leu Lys Pro Ala Lys Ala Ala Thr Arg Thr
      210          215          220
Ser Arg Gly Trp His Thr Thr Asp Leu Lys Tyr Asn Pro Ala Arg Val
      225          230          235          240
Glu Ala Phe His Arg Tyr Gly Thr Thr Val Asn Cys Ile Val Glu Glu
      245          250          255
Val Glu Ala Arg Ser Val Tyr Pro Tyr Asp Glu Phe Val Leu Ala Thr
      260          265          270
Gly Asp Phe Val Tyr Met Ser Pro Phe Tyr Gly Tyr Arg Asp Gly Ser
      275          280          285
His Gly Glu His Thr Ala Tyr Ala Ala Asp Arg Phe Arg Gln Val Asp
      290          295          300
Gly Tyr Tyr Glu Arg Asp Leu Ser Thr Gly Arg Arg Ala Ala Ala Pro
      305          310          315          320
Val Thr Arg Asn Leu Leu Thr Thr Pro Lys Phe Thr Val Gly Trp Asp
      325          330          335
Trp Ala Pro Lys Arg Pro Ser Val Cys Thr Leu Thr Lys Trp Arg Glu
      340          345          350
Val Asp Glu Met Leu Arg Ala Glu Tyr Gly Pro Ser Phe Arg Phe Ser
      355          360          365
Ser Ala Ala Leu Ser Thr Thr Phe Thr Ala Asn Arg Thr Glu Tyr Ala
      370          375          380

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Leu 385	Ser	Arg	Val	Asp 390	Leu	Ala	Asp	Cys	Val	Gly 395	Arg	Glu	Ala	Arg	Glu 400
Ala	Val	Asp	Arg	Ile 405	Phe	Leu	Arg	Arg	Tyr 410	Asn	Gly	Thr	His	Val	Lys 415
Val	Gly	Gln	Val	Gln 420	Tyr	Tyr	Leu	Ala 425	Thr	Gly	Gly	Phe	Leu	Ile	Ala 430
Tyr	Gln	Pro	Leu	Leu 435	Ser	Asn	Ala 440	Leu	Val	Glu	Leu	Tyr	Val	Arg	Glu 445
Leu	Val	Arg	Glu	Gln 450	Thr	Arg 455	Arg	Pro	Ala	Gly	Gly	Asp	Pro	Gly	Glu 460
Ala	Ala	Thr	Pro	Gly 465	Pro	Ser	Val	Asp 470	Pro	Pro	Ser	Val	Glu	Arg	Ile 480
Lys	Thr	Thr	Ser	Ser 485	Val	Glu	Phe	Ala 490	Arg	Leu	Gln	Phe	Thr	Tyr	Asp 495
His	Ile	Gln	Arg	His 500	Val	Asn	Asp	Met 505	Leu	Gly	Arg	Ile	Ala	Thr	Ala 510
Trp	Cys	Glu	Leu	Gln 515	Asn	Arg	Glu 520	Leu	Thr	Leu	Trp	Asn	Glu	Ala	Arg 525
Arg	Leu	Asn	Pro	Gly 530	Ala	Ile 535	Ala	Ser	Ala	Thr	Val	Gly	Arg	Arg	Val 540
Ser	Ala	Arg	Met	Leu 545	Gly 550	Asp	Val	Met	Ala	Val	Ser	Thr	Cys	Val	Pro 560
Val	Ala	Pro	Asp	Asn 565	Val	Ile	Met	Gln 570	Asn	Ser	Ile	Gly	Val	Ala	Ala 575
Arg	Pro	Gly	Thr	Cys 580	Tyr	Ser	Arg	Pro 585	Leu	Val	Ser	Phe	Arg	Tyr	Glu 590
Ala	Asp	Gly	Pro	Leu 595	Val	Glu	Gly 600	Gln	Leu	Gly	Glu	Asp	Asn	Glu	Ile 605
Arg	Leu	Glu	Arg	Asp 610	Ala	Leu	Glu 615	Pro	Cys	Thr	Val	Gly	His	Arg	Arg 620
Tyr	Phe	Thr	Phe	Gly 625	Ala 630	Gly	Tyr	Val	Tyr	Phe	Glu	Glu	Tyr	Ala	Tyr 640
Ser	His	Gln	Leu	Gly 645	Arg	Ala	Asp 650	Val	Thr	Thr	Val	Ser	Thr	Phe	Ile 655
Asn	Leu	Asn	Leu	Thr 660	Met	Leu	Glu 665	Asp	His	Glu	Phe	Val	Pro	Leu	Glu 670
Val	Tyr	Thr	Arg	Gln 675	Glu	Ile	Lys 680	Asp	Ser	Gly	Leu	Leu	Asp	Tyr	Thr 685
Glu	Val	Gln	Arg	Arg 690	Asn	Gln 695	Leu	His	Ala	Leu	Arg	Phe	Ala	Asp	Ile 700
Asp	Thr	Val	Ile	Lys 705	Ala 710	Asp	Ala	His	Ala	Ala	Leu	Phe	Ala	Gly	Leu 720
Tyr	Ser	Phe	Phe	Glu 725	Gly	Leu	Gly	Asp 730	Val	Gly	Arg	Ala	Val	Gly	Lys 735
Val	Val	Met	Gly	Ile 740	Val	Gly	Gly 745	Val	Val	Ser	Ala	Val	Ser	Gly	Val 750
Ser	Ser	Phe	Leu	Ser 755	Asn	Pro	Phe 760	Gly	Ala	Leu	Ala	Val	Gly	Leu	Leu 765
Val	Leu	Ala	Gly	Leu 770	Ala	Ala 775	Ala	Phe	Phe	Ala	Phe	Arg	Tyr	Val	Met 780
Arg	Leu	Gln	Arg	Asn 785	Pro 790	Met	Lys	Ala	Leu	Tyr 795	Pro	Leu	Thr	Thr	Lys 800
Glu	Leu	Lys	Ser	Asp 805	Gly	Ala	Pro	Leu	Ala	Gly	Gly	Gly	Glu	Asp	Gly 815

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Ala Glu Asp Phe Asp Glu Ala Lys Leu Ala Gln Ala Arg Glu Met Ile
 820 825 830

Arg Tyr Met Ala Leu Val Ser Ala Met Glu Arg Thr Glu His Lys Ala
 835 840 845

Arg Lys Lys Gly Thr Ser Ala Leu Leu Ser Ala Lys Val Thr Asp Ala
 850 855 860

Val Met Arg Lys Arg Ala Arg Pro Arg Tyr Ser Pro Leu Arg Asp Thr
 865 870 875 880

Asp Glu Glu Glu Leu
 885

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 33 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GCTGTTTCAGA TTGACTTAG AYMANMCNTG YCC

33

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 35 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GTGTACAAGA AGAACATCGT GCCNTAYATN TTYAA

35

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 23 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GTGTACAAGA AGAACATCGT GCC

23

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 32 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

AACATGTCTA CAATCTCACA RTTNACNGTN GT

32

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

AACATGTCTA CAATCTCACA

20

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(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 38 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

AATAACCTCT TTACGGCCCA AATTCARTWY GCNTAYGA 38

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 38 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CCAACGAGTG TGATGTCAGC CATTTAYGGN AARCCNGT 38

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CCAACGAGTG TGATGTCAGC C 21

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 38 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TGCTACTCGC GACCTCTAGT CACCTTYAAR TTYRTNAA 38

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

TGCTACTCGC GACCTCTAGT CACC 24

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 36 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

ACCGGAGTAC AGTTCCACTG TYTTAAARTC DATRTT 36

-continued

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TGTCACCTTG ACATGAGGCC A 21

(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 32 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

TTTGACCTGG AGACTATGTT YMGNGARTAY AA 32

(2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 33 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GCTCTGGGTG TAGTAGTTRT AYTCYCTRAA CAT 33

(2) INFORMATION FOR SEQ ID NO:38:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 32 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

TCTCGGAACA TGCTCTCCAG RTCRAAMACR TT 32

(2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 32 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

ACCTTCATCA AAAATCCCTT NGGNGGNATG YT 32

(2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 32 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

TGGACTTACA GGA CTGGAAC NACNGTNAAY TG 32

(2) INFORMATION FOR SEQ ID NO:41:

-continued

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 35 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

AGACCCGTGC CACTCTATGA RATHAGYCA ATGGA 35

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

AGACCCGTGC CACTCTATGA 20

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 30 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GTTCAACA ATCTTCATNG ARCTRAARCA 30

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

GTTCAACA ATCTTCAT 18

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 29 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

GTCAACGGAG TAGARAAYAC NTTYACNGA 29

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 35 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

ACTGGCTGGC TAAAGTACCT TTGAATRTTR TCNGT 35

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 23 base pairs

-continued

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

ACTGGCTGGC TAAAGTACCT TTG 23

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

TGCTGCTTCT GTCATACCGC G 21

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

TATTTGTTTG TGATTGCTGC T 21

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

GCGGTATGAC AGAAGCAGCA A 21

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

AACAAATATG AGATCCCCAG G 21

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

TCATCCCGAT CGGTGAACGT A 21

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

-continued

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

TTGTCAGTTA GACCTTCGAC G 21

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

CCCGTCGAAG GTCTAACTGA C 21

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

AGCCAACCAG TACTGTACTC T 21

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

TGATGGCGGA CTCTGTCAAG C 21

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

G TTCATACTT GTTGGTGATG G 21

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

GGGCTTGACA GAGTCCGCCA T 21

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

-continued

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

ACAAGTATGA ACTCCCGAGA C 21

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

ACCCCGTTGA CATTACCTT C 21

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

TCGTCTCTGT CAGTAAATGT G 21

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

CCACAGTATT CCTCCAACCA G 21

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

GGTACTTTAG CCAGCCGGTC A 21

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

Tyr Arg Lys Ile Ala Thr Ser Val Thr Val Tyr Arg Gly
 1 5 10

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

-continued

Ile Tyr Ala Glu Pro Gly Trp Phe Pro Gly Ile Tyr Arg Val Arg
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:66:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

Arg Tyr Phe Ser Gln Pro
1 5

(2) INFORMATION FOR SEQ ID NO:67:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

Val Thr Val Tyr Arg Gly
1 5

(2) INFORMATION FOR SEQ ID NO:68:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

Ala Ile Thr Asn Lys Tyr Glu
1 5

(2) INFORMATION FOR SEQ ID NO:69:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

Ser His Met Asp Ser Thr Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:70:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

Val Glu Asn Thr Phe Thr Asp
1 5

(2) INFORMATION FOR SEQ ID NO:71:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid

-continued

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

Thr Val Phe Leu Gln Pro Val
1 5

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

Thr Asp Asn Ile Gln Arg Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

Arg Gly Met Thr Glu Ala Ala
1 5

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

Pro Val Leu Tyr Ser Glu Pro
1 5

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

Arg Gly Leu Thr Glu Ser Ala
1 5

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

Pro Val Ile Tyr Ala Glu Pro
1 5

(2) INFORMATION FOR SEQ ID NO:77:

-continued

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 27 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

GCCTTTGAGA ATTCYAARTA YATHAAR

27

(2) INFORMATION FOR SEQ ID NO:78:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 27 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

GGGTTTGAGA ATTCYAARTA YATHAAR

27

(2) INFORMATION FOR SEQ ID NO:79:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 35 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

Thr Ala Ala Ala Gly Thr Ala Cys Ala Gly Cys Thr Cys Cys Thr
 1 5 10 15
 Gly Cys Cys Cys Gly Ala Ala Asn Ala Cys Arg Thr Thr Asn Ala Cys
 20 25 30
 Arg Cys Ala
 35

(2) INFORMATION FOR SEQ ID NO:80:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

TGTGGAACG GGAGCGTACA C

21

(2) INFORMATION FOR SEQ ID NO:81:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

TCAGACAAGA GTACGTGTCG G

21

(2) INFORMATION FOR SEQ ID NO:82:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

-continued

TACAGGTCGA CCGTAGATGG C 21

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

CGCCATTTC GTGACCGAGT G 21

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

TGATGAAGTA GTGTCGCAG G 21

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

GATGCCACCC AGGTCCGCCA C 21

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

GTGGCGGACC TGGGTGCAT C 21

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

CGTAGATCGC AGGGCACCTC C 21

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

GTCTCTCCCG CGAATACTTC T 21

-continued

(2) INFORMATION FOR SEQ ID NO:89:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

GAGGGCCTGC TGGAGGACGT G 21

(2) INFORMATION FOR SEQ ID NO:90:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

CGGTGGAGAA GCCGCAGGAT G 21

(2) INFORMATION FOR SEQ ID NO:91:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 3612 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 2..406
 (D) OTHER INFORMATION: /function=
 "Capsid/Maturation/Transport gene"

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 393..2927
 (D) OTHER INFORMATION: /function= "Glycoprotein B gene"

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 3057..3611
 (D) OTHER INFORMATION: /product= "DNA Polymerase"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

TGGGGGCATG TTTCCCATTC AAAAGATGAT GGTATCAGAG ATGATCTGGC CCAGCATAGA 60
 GCGGAAGGAC TGGATAGAGC CCAACTTCAA CCAGTTCTAT AGCTTTGAGA ATCAAGACAT 120
 AAACCATCTG CAAAAGAGAG CTTGGAATA TATCAGAGAG CTGGTATTAT CGGTTTCTCT 180
 GAACAACAGA ACTTGGGAGA GGGAGCTAAA AATACTTCTC ACGCCTCAGG GCTCACC GGG 240
 GTTTGAGGAA CCGAAACCCG CAGGACTCAC AACGGGGCTG TACCTAACAT TTGAGATATC 300
 TGCGCCCTTG GTGTTGGTGG ATAAAAAATA TGGCTGGATA TTAAAGACC TGTACGCCCT 360
 TCTGTACCAC CACCTGCAAC TGAGCAACCA CAATGACTCC CAGGTCTAGA TTGGCCACCC 420
 TGGGGACTGT CATCCTGTTG GTCTGCTTTT GCGCAGGCGC GCGCGACTCG AGGGGTGACA 480
 CCTTTCAGAC GTCCAGTTCC CCCACACCCC CAGGATCTTC CTCTAAGGCC CCCACCAAAC 540
 CTGGTGAGGA AGCATCTGGT CCTAAGAGTG TGGACTTTTA CCAGTTCAGA GTGTGTAGTG 600
 CATCGATCAC CGGGGAGCTT TTTCGGTTCA ACCTGGAGCA GACGTGCCCA GACACCAAAG 660
 ACAAGTACCA CCAAGAAGGA ATTTTACTGG TGTACAAAAA AAACATAGTG CCTCATATCT 720
 TTAAGGTGCG GCGCTATAGG AAAATTGCCA CCTCTGTCAC GGTCTACAGG GGCTTGACAG 780

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AGTCCGCCAT	CACCAACAAG	TATGAACTCC	CGAGACCCGT	GCCACTCTAT	GAGATAAGCC	840
ACATGGACAG	CACCTATCAG	TGCTTTAGTT	CCATGAAGGT	AAATGTCAAC	GGGGTAGAAA	900
ACACATTTAC	TGACAGAGAC	GATGTTAACA	CCACAGTATT	CCTCCAACCA	GTAGAGGGGC	960
TTACGGATAA	CATTCAAAGG	TACTTTAGCC	AGCCGGTCAT	CTACGCGGAA	CCCGGCTGGT	1020
TTCCCGGCAT	ATACAGAGTT	AGGACCACYG	TCAATTGCGA	GATAGTGGAC	ATGATAGCCA	1080
GGTCTGCTGA	ACCATACAAT	TACTTTGTCA	CGTCACTGGG	TGACACGGTG	GAAGTCTCCC	1140
CTTTTGTCTA	TAACGAATCC	TCATGCAGCA	CAACCCCCAG	CAACAAAAAT	GGCCTTAGCG	1200
TCCAAGTAGT	TCTCAACCAC	ACTGTGGTCA	CGTACTCTGA	CAGAGGAACC	AGTCCCCTC	1260
CCCAAAACAG	GATCTTTGTG	GAAACGGGAG	CGTACACGCT	TTCGTGGGCC	TCCGAGAGCA	1320
AGACCACGGC	CGTGTGTCCG	CTGGCACTGT	GGAAAACCTT	CCCGCGCTCC	ATCCAGACTA	1380
CCCACGAGGA	CAGCTTCCAC	TTTGTGGCCA	ACGAGATCAC	GGCCACCTTC	ACGGCTCCTC	1440
TAACGCCAGT	GGCCAACCTT	ACCGACACGT	ACTCTTGCT	GACCTCGGAT	ATCAACACCA	1500
CGCTTAACGC	CAGCAAGGCC	AAACTGGCGA	GCACTCACGT	CCCTAACGGG	ACGGTCCAGT	1560
ACTTCCACAC	AACAGGCGGA	CTCTATTTGG	TCTGGCAGCC	CATGTCCGCG	ATTAACCTGA	1620
CTCACGCTCA	GGGCGACAGC	GGGAACCCCA	CGTCATCGCC	GCCCCCTTCC	GCATCCCCCA	1680
TGACCACCTC	TGCCAGCCGC	AGAAAGAGAC	GGTCAGCCAG	TACCGCTGCT	GCCGGCGGCG	1740
GGGGGTCCAC	GGACAACCTG	TCTTACACGC	AGCTGCAGTT	TGCCTACGAC	AAACTGCGGG	1800
ATGGCATTAA	TCAGGTGTTA	GAAGAAGTCT	CCAGGGCATG	GTGTCGCGAG	CAGGTCAGGG	1860
ACAACCTAAT	GTGTACGAG	CTCAGTAAAA	TCAACCCAC	CAGCGTTATG	ACAGCCATCT	1920
ACGGTCGACC	TGTATCCGCC	AAGTTCGTAG	GAGACGCCAT	TTCCGTGACC	GAGTGCATTA	1980
ACGTGGACCA	GAGTCCGTA	AACATCCACA	AGAGCCTCAG	AACCAATAGT	AAGGACGTGT	2040
GTTACGCGCG	CCCCCTGGTG	ACGTTTAAGT	TTTTGAACAG	TTCCAACCTA	TTCAACGGCC	2100
AGCTGGGCGC	GCGCAATGAG	ATAATACTGA	CCAACAACCA	GGTGGAAC	TGCAAAGACA	2160
CCTGCGAACA	CTACTTCATC	ACCCGCAACG	AGACTCTGGT	GTATAAGGAC	TACGCGTACC	2220
TGCGCACTAT	AAACACCACT	GACATATCCA	CCCTGAACAC	TTTTATCGCC	CTGAATCTAT	2280
CCTTTATTCA	AAACATAGAC	TTCAAGGCCA	TCGAGCTGTA	CAGCAGTGCA	GAGAAACGAC	2340
TCGCGAGTAG	CGTGTTTGAC	CTGGAGACGA	TGTTTCAGGA	GTACAACCTAC	TACACACATC	2400
GTCTCGCGGG	TTTGCGCGAG	GATCTGGACA	ACACCATAGA	TATGAACAAG	GAGCGCTTCG	2460
TAAGGGACTT	GTCGGAGATA	GTGGCGGACC	TGGGTGGCAT	CGGAAAAACG	GTKGTGAACG	2520
TGGCCAGCAG	CGTGGTCACT	CTATGTGGCT	CATTGGTTAC	CGGATTCATA	AATTTTATTA	2580
AACACCCCTT	AGGTGGCATG	CTGATGATCA	TTATCGTTAT	AGCAATCATC	CTGATCATTT	2640
TTATGCTCAG	TCGCCGCACC	AATACCATAG	CCCAGGCGCC	GGTGAAGATG	ATCTACCCCG	2700
ACGTAGATCG	CAGGGCACCT	CCTAGCGGCG	GAGCCCCAAC	ACGGGAGGAA	ATCAAAAACA	2760
TCCTGTCTGG	AATGCACCAG	CTACAACAAG	AGGAGAGGCA	GAAGGCGGAT	GATYTGAAAA	2820
AAAGTACACC	CTCGGTGTTT	CAGCGTACCG	CAAACGGCCT	TCGTCAGCGT	CTGAGAGGAT	2880
ATAAACCTCT	GACTCAATCG	CTAGACATCA	GTCYGGAAAC	GGGGGAGTGA	CAGTGGATTC	2940
GAGGTTATTG	TTTGATGTAA	ATTTAGGAAA	CACGGCCCGC	CTCTGAAGCA	CCACATACAG	3000
ACTGCAGTTA	TCAACCCATC	TCGTTGCACA	CAGACACAAA	TTACCGTCCG	CAGATCATGG	3060
ATTTTTTCAA	TCCATTTATC	GACCCAACTC	GCGGAGGCCC	GAGAAACACT	GTGAGGCAAC	3120
CCACGCCGTC	ACAGTCGCCA	ACTGTCCCCT	CGGAGACAAG	AGTATGCAGG	CTTATACCGG	3180

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CCTGTTTCCA	AACCCCGGGG	CGACCCGGCG	TGGTTGCCGT	GGACACCACA	TTTCCACCCA	3240
CCTACTTCCA	GGGCCCCAAG	CGGGGAGAAG	TATTCGCGGG	AGAGACTGGG	TCTATCTGGA	3300
AAACAAGGCG	CGGACAGGCA	CGCAATGCTC	CTATGTCGCA	CCTCATATTC	CACGTATACG	3360
ACATCGTGGA	GACCACCTAC	ACGGCCGACC	GCTGCGAGGA	CGTGCCATTT	AGCTTCCAGA	3420
CTGATATCAT	TCCCAGCGGC	ACCGTCCTCA	AGCTGCTCGG	CAGAACACTA	GATGGCGCCA	3480
GTGTCTGCGT	GAACGTTTTT	AGGCAGCGCT	GCTACTTCTA	CACACTAGCA	CCCCAGGGGG	3540
TAAACCTGAC	CCACGTCCTC	CAGCAGGCCC	TCCAGGCTGG	CTTCGGTCGC	GCATCCTGCG	3600
GCTTCTCCAC	CG					3612

(2) INFORMATION FOR SEQ ID NO:92:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 3056 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

TGGGGGCATG	TTTCCCATT	AAAAGATGAT	GGTATCAGAG	ATGATCTGGC	CCAGCATAGA	60
GCGGAAGGAC	TGGATAGAGC	CCAACCTCAA	CCAGTTCTAT	AGCTTTGAGA	ATCAAGACAT	120
AAACCATCTG	CAAAAGAGAG	CTTGGGAATA	TATCAGAGAG	CTGGTATTAT	CGGTTTCTCT	180
GAACAACAGA	ACTTGGGAGA	GGGAGCTAAA	AATACTTCTC	ACGCCTCAGG	GCTCACCGGG	240
GTTTGAGGAA	CCGAAACCCG	CAGGACTCAC	AACGGGGCTG	TACCTAACAT	TTGAGATATC	300
TGCGCCCTTG	GTGTGGGTGG	ATAAAAAATA	TGGCTGGATA	TTTAAAGACC	TGTACGCCCT	360
TCTGTACCAC	CACCTGCAAC	TGAGCAACCA	CAATGACTCC	CAGGTCTAGA	TTGGCCACCC	420
TGGGGACTGT	CATCCTGTTG	GTCTGCTTTT	GCGCAGGCGC	GGCGCACTCG	AGGGGTGACA	480
CCTTTCAGAC	GTCCAGTTCC	CCCACACCCC	CAGGATCTTC	CTCTAAGGCC	CCCACCAAAC	540
CTGGTGAGGA	AGCATCTGGT	CCTAAGAGTG	TGGACTTTTA	CCAGTTCAGA	GTGTGTAGTG	600
CATCGATCAC	CGGGGAGCTT	TTTCGGTTCA	ACCTGGAGCA	GACGTGCCCA	GACACCAAAG	660
ACAAGTACCA	CCAAGAAGGA	ATTTTACTGG	TGTACAAAAA	AAACATAGTG	CCTCATATCT	720
TTAAGGTGCG	GCGCTATAGG	AAAATTGCCA	CCTCTGTAC	GGTCTACAGG	GGCTTGACAG	780
AGTCCGCCAT	CACCAACAAG	TATGAAGTCC	CGAGACCCGT	GCCACTCTAT	GAGATAAGCC	840
ACATGGACAG	CACCTATCAG	TGCTTTAGTT	CCATGAAGGT	AAATGTCAAC	GGGGTAGAAA	900
ACACATTTAC	TGACAGAGAC	GATGTTAACA	CCACAGTATT	CCTCCAACCA	GTAGAGGGGC	960
TTACGGATAA	CATTCAAAGG	TACTTTAGCC	AGCCGGTCAT	CTACGCGGAA	CCCGGCTGGT	1020
TTCCCGGCAT	ATACAGAGTT	AGGACCACYG	TCAATTGCGA	GATAGTGGAC	ATGATAGCCA	1080
GGTCTGCTGA	ACCATACAAT	TACTTTGTCA	CGTCACTGGG	TGACACGGTG	GAAGTCTCCC	1140
CTTTTGTCTA	TAACGAATCC	TCATGCAGCA	CAACCCCCAG	CAACAAAAAT	GGCCTTAGCG	1200
TCCAAGTAGT	TCTCAACCAC	ACTGTGGTCA	CGTACTCTGA	CAGAGGAACC	AGTCCCCTC	1260
CCCCAAAACAG	GATCTTTGTG	GAAACGGGAG	CGTACACGCT	TTCGTGGGCC	TCCGAGAGCA	1320
AGACCACGGC	CGTGTGTCCG	CTGGCACTGT	GGAAAACCTT	CCCGCGCTCC	ATCCAGACTA	1380
CCCACGAGGA	CAGCTTCCAC	TTTGTGGCCA	ACGAGATCAC	GGCCACCTTC	ACGGCTCCTC	1440
TAACGCCAGT	GGCCAACCTT	ACCGACACGT	ACTCTTGTCT	GACCTCGGAT	ATCAACACCA	1500
CGCTTAACGC	CAGCAAGGCC	AAACTGGCGA	GCACTCACGT	CCCTAACGGG	ACGGTCCAGT	1560
ACTTCCACAC	AACAGGCGGA	CTCTATTTGG	TCTGGCAGCC	CATGTCCGCG	ATTAACCTGA	1620

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CTCACGCTCA GGGCGACAGC GGGAAACCCCA CGTCATCGCC GCCCCCCTCC GCATCCCCCA 1680
TGACCACCTC TGCCAGCCGC AGAAAGAGAC GGTGAGCCAG TACCGCTGCT GCCGGCGGCG 1740
GGGGGTCCAC GGACAACCTG TCTTACACGC AGCTGCAGTT TGCCTACGAC AAAGTGCGGG 1800
ATGGCATTAA TCAGGTGTTA GAAGAACTCT CCAGGGCATG GTGTCGCGAG CAGGTCAGGG 1860
ACAACCTAAT GTGTACGAG CTCAGTAAAA TCAACCCAC CAGCGTTATG ACAGCCATCT 1920
ACGGTCGACC TGTATCCGCC AAGTTCGTAG GAGACGCCAT TTCCGTGACC GAGTGCATTA 1980
ACGTGGACCA GAGTCCGTA AACATCCACA AGAGCCTCAG AACCAATAGT AAGGACGTGT 2040
GTTACGCGCG CCCCTGGTG ACGTTTAAGT TTTTGAACAG TTCCAACCTA TTCACCGGCC 2100
AGCTGGGCGC GCGCAATGAG ATAATACTGA CCAACAACCA GGTGGAAACC TGCAAAGACA 2160
CCTGCGAACA CTACTTCATC ACCCGCAACG AGACTCTGGT GTATAAGGAC TACGCGTACC 2220
TGCGCACTAT AAACACCACT GACATATCCA CCCTGAACAC TTTTATCGCC CTGAATCTAT 2280
CCTTTATTCA AAACATAGAC TTCAAGGCCA TCGAGCTGTA CAGCAGTGCA GAGAAACGAC 2340
TCGCGAGTAG CGTGTGTTGAC CTGGAGACGA TGTTGAGGGA GTACAACTAC TACACACATC 2400
GTCTCGCGGG TTTGCGCGAG GATCTGGACA ACACCATAGA TATGAACAAG GAGCGCTTCG 2460
TAAGGGACTT GTCGGAGATA GTGGCGGACC TGGGTGGCAT CGGAAAAACG GTKGTGAACG 2520
TGGCCAGCAG CGTGGTCACT CTATGTGGCT CATTGGTTAC CGGATTCATA AATTTTATTA 2580
AACACCCCTC AGGTGGCATG CTGATGATCA TTATCGTTAT AGCAATCATC CTGATCATTT 2640
TTATGCTCAG TCGCCGCACC AATACCATAG CCCAGGCGCC GGTGAAGATG ATCTACCCCG 2700
ACGTAGATCG CAGGACACCT CCTAGCGGCG GAGCCCCAAC ACGGGAGGAA ATCAAAAACA 2760
TCCTGTGGG AATGCACCAG CTACAACAAG AGGAGAGGCA GAAGGCGGAT GATYTGAAAA 2820
AAAGTACACC CTCGGTGTTT CAGCGTACCG CAAACGGCCT TCGTCAGCGT CTGAGAGGAT 2880
ATAAACCTCT GACTCAATCG CTAGACATCA GTCYGGAAAC GGGGGAGTGA CAGTGGATTC 2940
GAGGTTATTG TTTGATGTAA ATTTAGGAAA CACGGCCCGC CTCTGAAGCA CCACATACAG 3000
ACTGCAGTTA TCAACCCTAC TCGTTGCACA CAGACACAAA TTACCGTCCG CAGATC 3056

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(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 135 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

```

Gly Gly Met Phe Pro Ile Gln Lys Met Met Val Ser Glu Met Ile Trp
1           5           10           15
Pro Ser Ile Glu Arg Lys Asp Trp Ile Glu Pro Asn Phe Asn Gln Phe
20          25          30
Tyr Ser Phe Glu Asn Gln Asp Ile Asn His Leu Gln Lys Arg Ala Trp
35          40          45
Glu Tyr Ile Arg Glu Leu Val Leu Ser Val Ser Leu Asn Asn Arg Thr
50          55          60
Trp Glu Arg Glu Leu Lys Ile Leu Leu Thr Pro Gln Gly Ser Pro Gly
65          70          75          80
Phe Glu Glu Pro Lys Pro Ala Gly Leu Thr Thr Gly Leu Tyr Leu Thr
85          90          95
Phe Glu Ile Ser Ala Pro Leu Val Leu Val Asp Lys Lys Tyr Gly Trp
100         105         110

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Ile Phe Lys Asp Leu Tyr Ala Leu Leu Tyr His His Leu Gln Leu Ser
 115 120 125

Asn His Asn Asp Ser Gln Val
 130 135

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 845 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 841
- (D) OTHER INFORMATION: /note= "Proline or Leucine depending on codon"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

Met Thr Pro Arg Ser Arg Leu Ala Thr Leu Gly Thr Val Ile Leu Leu
 1 5 10 15

Val Cys Phe Cys Ala Gly Ala Ala His Ser Arg Gly Asp Thr Phe Gln
 20 25 30

Thr Ser Ser Ser Pro Thr Pro Pro Gly Ser Ser Ser Lys Ala Pro Thr
 35 40 45

Lys Pro Gly Glu Glu Ala Ser Gly Pro Lys Ser Val Asp Phe Tyr Gln
 50 55 60

Phe Arg Val Cys Ser Ala Ser Ile Thr Gly Glu Leu Phe Arg Phe Asn
 65 70 75 80

Leu Glu Gln Thr Cys Pro Asp Thr Lys Asp Lys Tyr His Gln Glu Gly
 85 90 95

Ile Leu Leu Val Tyr Lys Lys Asn Ile Val Pro His Ile Phe Lys Val
 100 105 110

Arg Arg Tyr Arg Lys Ile Ala Thr Ser Val Thr Val Tyr Arg Gly Leu
 115 120 125

Thr Glu Ser Ala Ile Thr Asn Lys Tyr Glu Leu Pro Arg Pro Val Pro
 130 135 140

Leu Tyr Glu Ile Ser His Met Asp Ser Thr Tyr Gln Cys Phe Ser Ser
 145 150 155 160

Met Lys Val Asn Val Asn Gly Val Glu Asn Thr Phe Thr Asp Arg Asp
 165 170 175

Asp Val Asn Thr Thr Val Phe Leu Gln Pro Val Glu Gly Leu Thr Asp
 180 185 190

Asn Ile Gln Arg Tyr Phe Ser Gln Pro Val Ile Tyr Ala Glu Pro Gly
 195 200 205

Trp Phe Pro Gly Ile Tyr Arg Val Arg Thr Thr Val Asn Cys Glu Ile
 210 215 220

Val Asp Met Ile Ala Arg Ser Ala Glu Pro Tyr Asn Tyr Phe Val Thr
 225 230 235 240

Ser Leu Gly Asp Thr Val Glu Val Ser Pro Phe Cys Tyr Asn Glu Ser
 245 250 255

Ser Cys Ser Thr Thr Pro Ser Asn Lys Asn Gly Leu Ser Val Gln Val
 260 265 270

Val Leu Asn His Thr Val Val Thr Tyr Ser Asp Arg Gly Thr Ser Pro
 275 280 285

Thr Pro Gln Asn Arg Ile Phe Val Glu Thr Gly Ala Tyr Thr Leu Ser
 290 295 300

-continued

Trp Ala Ser Glu Ser Lys Thr Thr Ala Val Cys Pro Leu Ala Leu Trp
 305 310 315 320
 Lys Thr Phe Pro Arg Ser Ile Gln Thr Thr His Glu Asp Ser Phe His
 325 330 335
 Phe Val Ala Asn Glu Ile Thr Ala Thr Phe Thr Ala Pro Leu Thr Pro
 340 345 350
 Val Ala Asn Phe Thr Asp Thr Tyr Ser Cys Leu Thr Ser Asp Ile Asn
 355 360 365
 Thr Thr Leu Asn Ala Ser Lys Ala Lys Leu Ala Ser Thr His Val Pro
 370 375 380
 Asn Gly Thr Val Gln Tyr Phe His Thr Thr Gly Gly Leu Tyr Leu Val
 385 390 395 400
 Trp Gln Pro Met Ser Ala Ile Asn Leu Thr His Ala Gln Gly Asp Ser
 405 410 415
 Gly Asn Pro Thr Ser Ser Pro Pro Pro Ser Ala Ser Pro Met Thr Thr
 420 425 430
 Ser Ala Ser Arg Arg Lys Arg Arg Ser Ala Ser Thr Ala Ala Ala Gly
 435 440 445
 Gly Gly Gly Ser Thr Asp Asn Leu Ser Tyr Thr Gln Leu Gln Phe Ala
 450 455 460
 Tyr Asp Lys Leu Arg Asp Gly Ile Asn Gln Val Leu Glu Glu Leu Ser
 465 470 475 480
 Arg Ala Trp Cys Arg Glu Gln Val Arg Asp Asn Leu Met Trp Tyr Glu
 485 490 495
 Leu Ser Lys Ile Asn Pro Thr Ser Val Met Thr Ala Ile Tyr Gly Arg
 500 505 510
 Pro Val Ser Ala Lys Phe Val Gly Asp Ala Ile Ser Val Thr Glu Cys
 515 520 525
 Ile Asn Val Asp Gln Ser Ser Val Asn Ile His Lys Ser Leu Arg Thr
 530 535 540
 Asn Ser Lys Asp Val Cys Tyr Ala Arg Pro Leu Val Thr Phe Lys Phe
 545 550 555 560
 Leu Asn Ser Ser Asn Leu Phe Thr Gly Gln Leu Gly Ala Arg Asn Glu
 565 570 575
 Ile Ile Leu Thr Asn Asn Gln Val Glu Thr Cys Lys Asp Thr Cys Glu
 580 585 590
 His Tyr Phe Ile Thr Arg Asn Glu Thr Leu Val Tyr Lys Asp Tyr Ala
 595 600 605
 Tyr Leu Arg Thr Ile Asn Thr Thr Asp Ile Ser Thr Leu Asn Thr Phe
 610 615 620
 Ile Ala Leu Asn Leu Ser Phe Ile Gln Asn Ile Asp Phe Lys Ala Ile
 625 630 635 640
 Glu Leu Tyr Ser Ser Ala Glu Lys Arg Leu Ala Ser Ser Val Phe Asp
 645 650 655
 Leu Glu Thr Met Phe Arg Glu Tyr Asn Tyr Tyr Thr His Arg Leu Ala
 660 665 670
 Gly Leu Arg Glu Asp Leu Asp Asn Thr Ile Asp Met Asn Lys Glu Arg
 675 680 685
 Phe Val Arg Asp Leu Ser Glu Ile Val Ala Asp Leu Gly Gly Ile Gly
 690 695 700
 Lys Thr Val Val Asn Val Ala Ser Ser Val Val Thr Leu Cys Gly Ser
 705 710 715 720
 Leu Val Thr Gly Phe Ile Asn Phe Ile Lys His Pro Leu Gly Gly Met

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(A) NAME/KEY: CDS
(B) LOCATION: 1..384

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

GTG TAC AAG AAG AAC ATC GTG CCT AAC ATG TTC AAG GTA CGC AGG TAC	48
Val Tyr Lys Lys Asn Ile Val Pro Asn Met Phe Lys Val Arg Arg Tyr	
1 5 10 15	
AGA AAA GTA GCA ACG CCT GTC ACA CTC TAC CGC GGT ATG ACA GAC GCA	96
Arg Lys Val Ala Thr Pro Val Thr Leu Tyr Arg Gly Met Thr Asp Ala	
20 25 30	
GCA ATA ACT AAC AAA TAT GAA ATT CCC AGA CCC GTA CCA CTA TAC GAG	144
Ala Ile Thr Asn Lys Tyr Glu Ile Pro Arg Pro Val Pro Leu Tyr Glu	
35 40 45	
ATC AGT CAC ATG GAC AGC ACC TAC CAG TGC TTT AGT TCC ATG AAA ATT	192
Ile Ser His Met Asp Ser Thr Tyr Gln Cys Phe Ser Ser Met Lys Ile	
50 55 60	
GTA GTG AAC GGA GTC GAA AAC ACG TTC ACC GGT CGG GAT GAC GTA AAC	240
Val Val Asn Gly Val Glu Asn Thr Phe Thr Gly Arg Asp Asp Val Asn	
65 70 75 80	
AAA AGC GTA TTT CTC CAG CCA GTC GAA GGT CTA ACT GAC AAC ATA AAG	288
Lys Ser Val Phe Leu Gln Pro Val Glu Gly Leu Thr Asp Asn Ile Lys	
85 90 95	
AGA TAC TTT AGC CAG CCA GTG CTA TAT TCT GAA CCC GGA TGG TTT CCA	336
Arg Tyr Phe Ser Gln Pro Val Leu Tyr Ser Glu Pro Gly Trp Phe Pro	
100 105 110	
GGT ATC TAC AGG GTT AGG ACA ACA GTT AAT TGT GAG ATT GTA GAC ATG	384
Gly Ile Tyr Arg Val Arg Thr Thr Val Asn Cys Glu Ile Val Asp Met	
115 120 125	
TT	386

(2) INFORMATION FOR SEQ ID NO:97:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 128 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

Val Tyr Lys Lys Asn Ile Val Pro Asn Met Phe Lys Val Arg Arg Tyr
1 5 10 15
Arg Lys Val Ala Thr Pro Val Thr Leu Tyr Arg Gly Met Thr Asp Ala
20 25 30
Ala Ile Thr Asn Lys Tyr Glu Ile Pro Arg Pro Val Pro Leu Tyr Glu
35 40 45
Ile Ser His Met Asp Ser Thr Tyr Gln Cys Phe Ser Ser Met Lys Ile
50 55 60
Val Val Asn Gly Val Glu Asn Thr Phe Thr Gly Arg Asp Asp Val Asn
65 70 75 80
Lys Ser Val Phe Leu Gln Pro Val Glu Gly Leu Thr Asp Asn Ile Lys
85 90 95
Arg Tyr Phe Ser Gln Pro Val Leu Tyr Ser Glu Pro Gly Trp Phe Pro
100 105 110
Gly Ile Tyr Arg Val Arg Thr Thr Val Asn Cys Glu Ile Val Asp Met
115 120 125

(2) INFORMATION FOR SEQ ID NO:98:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 26 base pairs

-continued

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

ATGTTTCAGGG AGTACAACCTA CTACAC

26

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

Ile Tyr Ala Glu Pro Gly Trp Phe Pro Gly Ile Tyr Arg Val Arg Thr
1 5 10 15
Thr Val Asn Cys Glu
20

(2) INFORMATION FOR SEQ ID NO:100:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

Val Leu Glu Glu Leu Ser Arg Ala Trp Cys Arg Glu Gln Val Arg Asp
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:101:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

Met Thr Pro Arg Ser Arg Leu Ala Thr Leu Gly Thr Val Ile Leu Leu
1 5 10 15
Val Cys Phe Cys Ala Gly Ala Ala His Ser Arg Gly Asp Thr Phe Gln
20 25 30
Thr Ser Ser Ser Pro Thr Pro Pro Gly Ser Ser Ser Ser Lys Ala Pro
35 40 45

(2) INFORMATION FOR SEQ ID NO:102:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

Met Val Pro Asn Lys His Leu Leu Leu Ile Leu Ser Phe Ser Thr Ala
1 5 10 15
Cys Gly Gln Thr Thr Pro Thr Thr Ala Val Glu Lys Asn Lys Thr Gln
20 25 30
Ala Ile Tyr Gln Glu Tyr Phe Lys Tyr Arg
35 40

-continued

(2) INFORMATION FOR SEQ ID NO:103:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 45 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

```
Met Tyr Tyr Lys Thr Ile Leu Phe Phe Ala Leu Ile Lys Val Cys Ser
1           5           10           15
Phe Asn Gln Thr Thr Thr His Ser Thr Thr Thr Ser Pro Ser Ile Ser
          20           25           30
Ser Thr Thr Ser Ser Thr Thr Thr Ser Thr Ser Lys Pro
      35           40           45
```

(2) INFORMATION FOR SEQ ID NO:104:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 49 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

```
Met Tyr Pro Thr Val Lys Ser Met Arg Val Ala His Leu Thr Asn Leu
1           5           10           15
Leu Thr Leu Leu Cys Leu Leu Cys His Thr His Leu Tyr Val Cys Gln
          20           25           30
Pro Thr Thr Leu Arg Gln Pro Ser Asp Met Thr Pro Ala Gln Asp Ala
      35           40           45
Pro
```

(2) INFORMATION FOR SEQ ID NO:105:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 48 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

```
Met Thr Arg Arg Arg Val Leu Ser Val Val Val Leu Leu Ala Ala Leu
1           5           10           15
Ala Cys Arg Leu Gly Ala Gln Thr Pro Glu Gln Pro Ala Pro Pro Ala
          20           25           30
Thr Thr Val Gln Pro Thr Ala Thr Arg Gln Gln Leu Ser Val Val Val
      35           40           45
```

(2) INFORMATION FOR SEQ ID NO:106:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

```
Cys Ala Gly Ala Ala His Ser Arg Gly Asp Thr Phe Gln Thr Ser Ser
1           5           10           15
Ser Pro Thr
```

(2) INFORMATION FOR SEQ ID NO:107:

-continued

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

Asn Ile Met Glu Ile Leu Arg Gly Asp Phe Ser Ser Ala Asn Asn Arg
 1 5 10 15

Asp Asn

(2) INFORMATION FOR SEQ ID NO:108:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

Ser Ser Thr Ser Tyr Asn Arg Gly Asp Ser Thr Phe Glu Ser Lys Ser
 1 5 10 15

Tyr Lys

(2) INFORMATION FOR SEQ ID NO:109:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

Ala Leu Gly Gly Asp Val Glu Lys Arg Gly Asp Arg Glu Glu Ala His
 1 5 10 15

Val Pro Phe Phe
 20

(2) INFORMATION FOR SEQ ID NO:110:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

Cys Gln Ala Gly Thr Phe Ala Leu Arg Gly Asp Ser Thr Phe Glu Glu
 1 5 10 15

Ser Lys Ser

(2) INFORMATION FOR SEQ ID NO:111:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

Ile Thr Val Tyr Ala Val Thr Gly Arg Gly Asp Ser Pro Ala Ser Ser
 1 5 10 15

Lys Pro Ile Ser
 20

-continued

(2) INFORMATION FOR SEQ ID NO:112:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:

Cys Glu Val Val Thr Gly Ser Pro Arg Gly Asp Ser Gln Ser Ser Trp
 1 5 10 15
 Lys Ser Val Gly
 20

(2) INFORMATION FOR SEQ ID NO:113:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:

Cys Lys Pro Gln Val Thr Arg Gly Asp Val Phe Thr Met Pro Glu Asp
 1 5 10 15
 Glu Tyr

What is claimed is:

1. An isolated polynucleotide comprising a sequence as set forth as nucleotides 36 to 354 of SEQ. ID NO:1 or SEQ. ID NO:3.

2. An isolated polynucleotide comprising a sequence selected from the group consisting of: SEQ. ID NO:41, SEQ. ID NO:43, SEQ. ID NO:45 and SEQ. ID NO:46.

3. An isolated polynucleotide comprising a sequence set forth in a member of the group consisting of nucleotides 36 to 354 inclusive of SEQ. ID NO:1, nucleotides 36 to 354 inclusive of SEQ ID NO:3, nucleotides 36 to 354 inclusive of SEQ ID NO:92 and SEQ. ID NO:96.

4. An isolated or non-naturally occurring polynucleotide encoding a polypeptide comprising a sequence as set forth in a member of the group consisting of amino acids 13 to 118 inclusive of SEQ ID NO:2, amino acids 13 to 118 inclusive of SEQ ID NO:4, amino acids 13 to 118 inclusive of SEQ ID NO:97, and SEQ ID NO: 94.

5. A recombinant cloning or expression vector comprising the polynucleotide of claim 4.

6. A host cell transformed by the polynucleotide of claim 4.

7. An oligonucleotide selected from the group consisting of SEQ. ID NOS:24-63, SEQ. ID NOS.77-78, and SEQ. ID NOS:80-90.

8. An isolated polynucleotide, where said polynucleotide is capable of hybridizing under conditions of high stringency with a second polynucleotide comprising a sequence selected from the group consisting of SEQ. ID NOS:1, 3, 92, and 94, and their respective complementary sequences, but is not capable of hybridizing under conditions of high stringency with a polynucleotide having a sequence of any of SEQ. ID NOS:5-13.

9. The isolated polynucleotide of claim 8, the nucleotide sequence of which is contained in the genome of a naturally occurring virus.

10. A monoclonal or isolated polyclonal antibody specific for a Glycoprotein B polypeptide encoded in said encoding region of the polynucleotide of claim 1.

11. A monoclonal or isolated polyclonal antibody specific for the polypeptide encoded by the polynucleotide of claim 4.

12. A diagnostic kit for detecting a herpes virus polynucleotide in a biological sample, comprising a reagent in suitable packaging, wherein the reagent comprises the polynucleotide of claim 3.

13. A diagnostic kit for detecting a herpes virus polypeptide present in a biological sample, comprising a reagent in suitable packaging, wherein the reagent comprises the antibody of claim 11.

14. A method of inhibiting attachment of a herpes virus to a cell, comprising contacting the cell with a polypeptide encoded by the polynucleotide of claim 4, wherein said polypeptide comprises an arginine-glycine-aspartic acid sequence.

15. A method of detecting infection of an individual by a herpes virus, comprising the steps of:

- contacting antibody from a sample obtained from the individual with the polypeptide encoded by the polynucleotide of claim 4 under conditions that permit the formation of a stable antigen-antibody complex; and
- detecting said stable complexes formed in step a), if any.

16. A method of detecting infection of an individual by a herpes virus, comprising the steps of:

- contacting a polypeptide from a sample obtained from the individual with the antibody of claim 11 under conditions that permit the formation of a stable antigen-antibody complex; and
- detecting said stable complexes formed in step a), if any.

17. A method of producing a Glycoprotein B polypeptide, comprising expressing the polynucleotide of claim 4 in a eukaryotic cell.

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